REVIEW

Advances of fluorescent DNA nanostructures in biomedical applications

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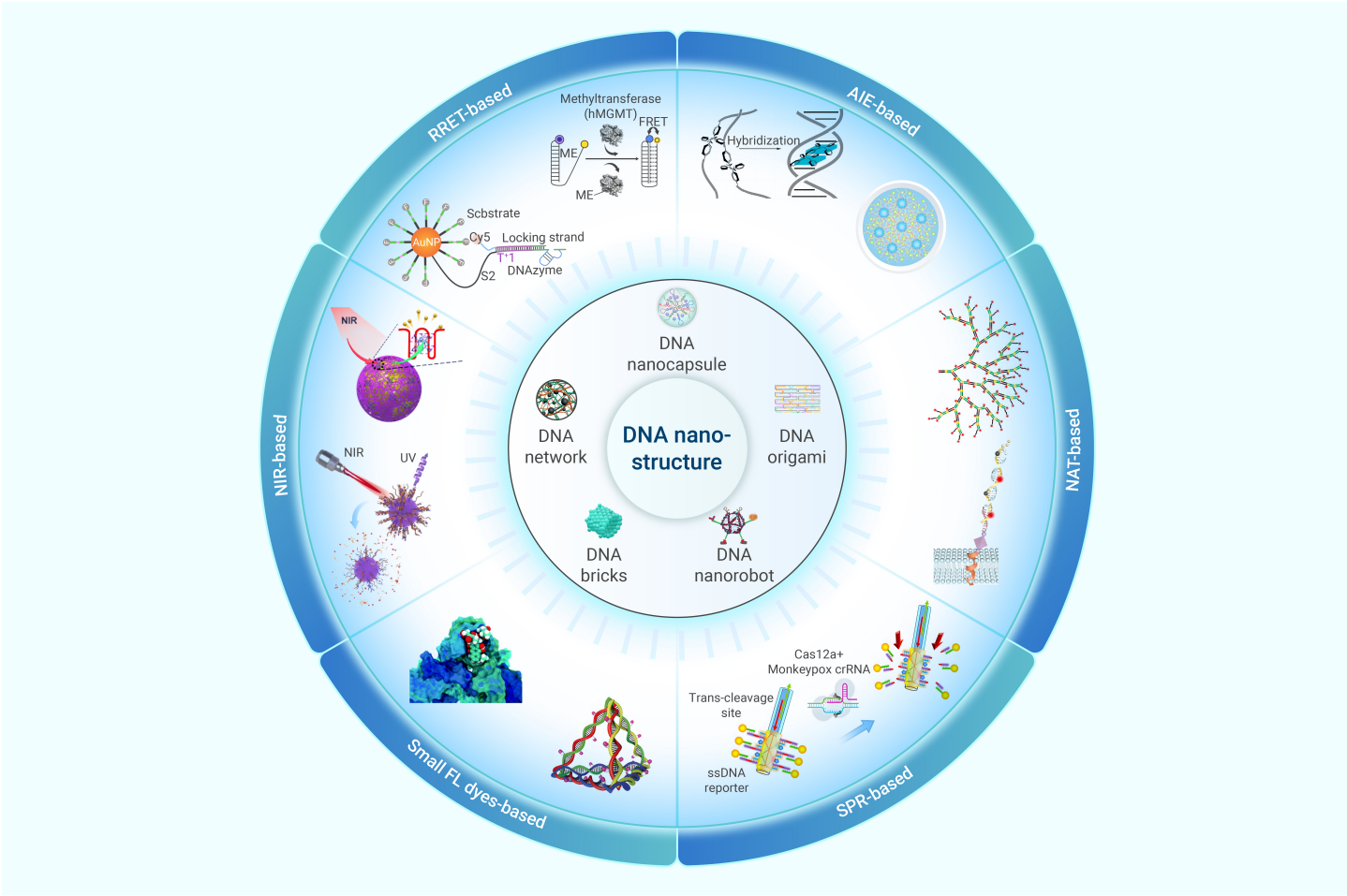
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Advances of fluorescent DNA nanostructures (FDNs) in biomedical applications are reviewed.
- Various DNA nanostructures are summarized.
- Remaining challenges and key research directions for enlarging biological applications of FDNs are proposed.

REVIEW

Advances of fluorescent DNA nanostructures in biomedical applications

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With the rapid development of DNA nanotechnology, the emergence of fluorescent DNA nanostructures (FDNs) has enlarged the biological applications. FDNs have great advantages of precise localization and real-time tracing in bioimaging. In this review, the recent biomedical developments of FDNs have been reviewed, including the design of FDNs, and the corresponding applications on biomarker sensing, bioimaging, cancer diagnosis and therapy. Firstly, the development of DNA nanostructures and the corresponding DNA-based nanomaterials were briefly introduced. Simultaneously, to make a better demonstration, the background and theory of the fluorescence detections were briefly introduced. Thereafter, the synthetic strategies of DNA nanostructure were summarized and classified, which facilitated the multiple functionalizations for sensing and bioimaging. Subsequently, the biomedical applications of FDNs are comprehensively summarized based on different detection strategies, including fluorescence resonance energy transfer (FRET), nucleic acid amplification (NAT), aggregation-induced emission (AIE), near-infrared (NIR)-photoactivation, small fluorescent dyes loading, and surface plasmon resonance (SPR) technologies. Finally, an insight into the challenges and future perspectives is provided. As reviewed, FDNs are important tools in precision medicine, showing great potential in both *in vivo* and *in vitro* cancer diagnosis and treatments. Undoubtedly, FDN-based technology is a promising strategy for constructing versatile nanodevices in biological applications and will excel in human healthcare.

INTRODUCTION

As an essential family of biological macromolecules, deoxyribonucleic acid (DNA) plays an indispensable role in storing and disseminating genetic information in living systems.[1] Based on the precise Watson-Crick base pairing, the DNA-based assembly has triggered nanotechnology into a more predictable dimension.[2] The first proposal of DNA nanotechnology was demonstrated by Seeman and coworkers in the 1980s. A DNA four-way junction was designed in this nanostructure through the complement of sticky ends with single-stranded overhangs. Thereafter, diverse DNA conformations have been employed upon precise design and user-defined organizations. More significantly, with the development of DNA manipulation,[3] considerable DNA-based rigid motifs have been prepared to construct different dimensional nanostructures. These DNA nanostructures have exhibited great potential in multiple biological applications. Compared to their traditional detection strategies, the highly predictable interactions and thermodynamics of DNA nanostructures have endowed DNA-based sensors with great advantages for biological applications. Upon precise designs of DNA nanostructures, the spatiotemporally controlled predictable, programmable and addressable biosensing platforms can be constructed with nanometer precision. Therefore, compared to traditional sensors, the DNA-based detection technologies would exhibit more outstanding performance for biological applications. For example, DNA-based nanostructures can be designed to act as biomolecular scaffolds or nanoparticle templates[4-8] for obtaining DNA tile assemblies,[9,10] DNA origami assemblies,[11-14] and DNA dynamic nanomechanical devices.[15-18] Consequently, a variety of DNA-based nanostructures have been prepared for various biological applications, owing to their inherent physiological functions, biocompatibility,[19-21] and biodegradability.[22-25] Furthermore, the development of DNA nanotechnology has been promoted by the increasing demand. This in turn facilitates the exploitation of new functional materials to meet huge biological and medical-related applications such as tissue engineering and therapeutics. Therefore, DNA nanotechnology has gradually become a booming field in materials science and biological engineering.

In spite of the burgeoning achievements in DNA technology, there are still several challenges that need to be addressed for biosensing developments. Firstly, the design and synthesis of complex structures with precise control over shape and functionality have been pursued and the requirements continue to be increased. In this regard, some softwares of computational modeling and simulation techniques have been proposed to simplify the process, which makes the structure more accurate. For example, DNA origami have been developed to create highly complex and precise DNA nanostructures. Additionally, external stimuli, such as light or temperature, have been adopted to control the assembly and disassembly of DNA nanostructures. Secondly, despite the low-cost of DNA strands, the cost can still be greatly increased upon introducing probe-labeled strands and tool-enzymes into the DNA-based biosensors. One possible solution to the problem is to employ label-free methods and enzyme-free methods for constructing DNA nanostructure devices. However, the further efforts are still encouraged for developing low-cost strategies. Thirdly, polymerase chain reaction has been frequently used to improve the detection sensitivity, while the complex temperature control and specialized instruments limited the applications. Accordingly, nucleic acid isothermal amplification nanoplatforms have been constructed for sensitive detection and bio-imaging, while more simple amplified techniques are further encouraged. Consequently, the DNA nanotechnology is still confined at the infant stage for practical applications. With the rapid development of DNA synthetic techniques, advanced functional materials and signal output strategies, DNA-based biosensors will definitely reach a new level and exhibit surprising magic performance in biomedical contexts.

Especially, fluorescence spectroscopy has unique merits of high spatial and temporal resolution, exhibiting great potential in biological and biomedical applications.[26,27] Based on fluorescent signals, a variety of detection strategies have been evoked, including fluorescence spectroscopy for solution-based assays, microscopy for cell imaging, flow cytometry for high-throughput analysis of single cells, and *in vivo* imaging. During these processes, fluorophores in DNA nanostructures played an important role in obtaining signals. They can be used as molecular transducers upon transfer of energy from chemical, physical, or biological events into light. This enabled the subsequent detection and analysis for interpreting otherwise inaccessible information by fluorescent DNA nanostructures (FDNs). FDNs hold tremendous promise in enhancing the sensitivity and versatility of DNA nanostructure-based methods, showing great potential in cancer diagnosis. In particular, structure-defining traits of FDNs, including size, shape, high surface area, and unique optical properties, have enabled the development of cancer diagnosis and treatments. Consequently, the development of fluorescent DNA nanotechnology would exhibit significant potential for biomedical applications.

In this review, fluorescent DNA nanotechnology strategies and the corresponding biomedical applications have been summarized based on fluorescence resonance energy transfer (FRET), nucleic acid amplification (NAT), aggregation-induced emission (AIE), near-infrared (NIR)-photoactivation, small fluorescent dyes loading, and surface plasmon resonance (SPR) technologies. In addition, based on the design and construction of DNA nanostructures, detailed applications have been introduced for biomarker sensing, bioimaging, cancer diagnosis and therapy.

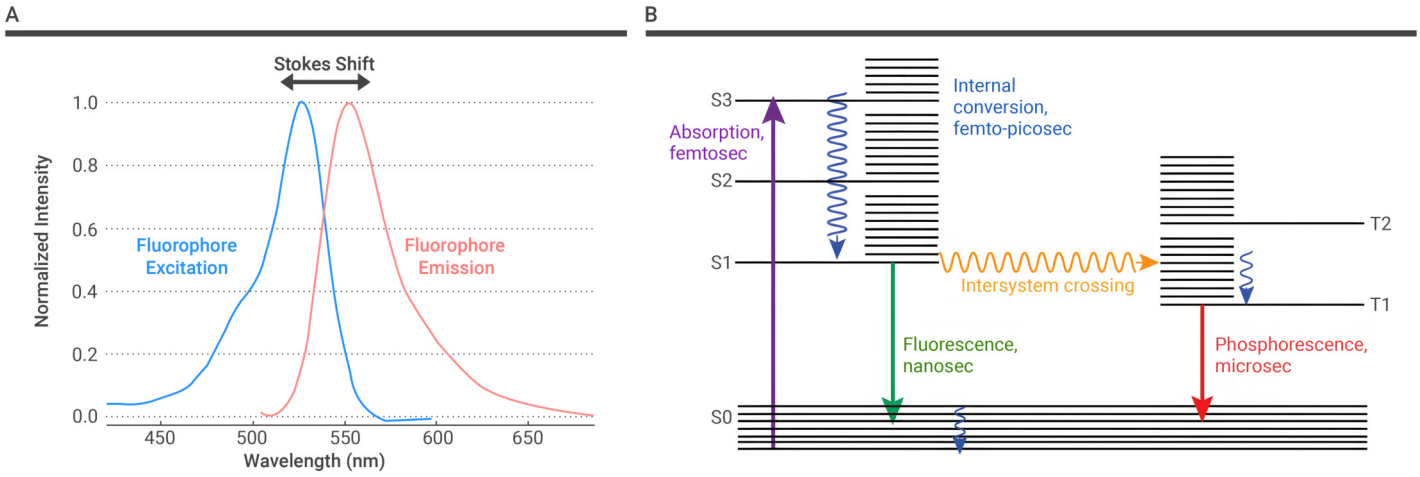

Figure 1. Principle of fluorescence detection (A) Molecular fluorescence spectrum illustrating the broadening of the spectral lines due to the presence of vibrational energy levels, and the Stokes shift between the excitation and emission maxima. Adapted with permission.[33] Copyright © 2015, American Chemical Society. (B) Jablonski diagram including typical time scales of photophysical processes for organic molecules. Adapted with permission.[34] Copyright © 2010, American Chemical Society.

FLUORESCENCE DETECTION: BACKGROUND AND THEORY

At present, the clinical cancer diagnosis primarily relies on imaging or the morphological analysis of corresponding cells (cytology) or the suspected diseased tissues (histopathology). There are many imaging techniques have been applied to cancer diagnosis, including X-ray, mammography, computed tomography, magnetic resonance imaging (MRI), endoscopy, and ultrasound. However, with the relatively low sensitivity, the differentiation of benign and malignant lesions could be somehow limited, which inspired further improvements of the imaging techniques.[28,29] Consequently, given the significant demands for early diagnosis in cancer latency, the sensitive detection methods are urgently required. Fortunately, with the development of nanoscience, quite a lot of nanomaterials have been constructed for addressing sensitive, efficient and biocompatible methods for diagnosis.[30,31] Especially, based on signals of fluorescence,[32] fluorescent nanomaterials have exhibited great advantages in sensitive detections, being beneficial for cancer diagnosis.

Fluorescence is an optical phenomenon, where the absorption of photons at one wavelength results in another emission at a longer wavelength. As shown in Figure 1A, upon vibrational relaxation, the energy between the absorbed and emitted photons could be lost, and a Stokes shift would be finally induced by these energy differences.[33] As illustrated in Figure 1B, the process of fluorescence could be clearly described by a typical Jablonski diagram.[34] Briefly, in the first stage of the excitation, the absorption of light initiates the promotion of an electron from the ground state to the excited state. Once excited, the absorbed energy may be released through several photophysical events, including both radiative and nonradiative emission. During this course, vibrational relaxation is often the first route of energy dissipation, which may be followed by internal conversion, and intersystem crossing (from a singlet to a triplet state). Consequently, subsequent phosphorescence or fluorescence would be emitted through the release of a photon, along with the returning of excited electrons to the ground state and emitting energy.[35] Based on different photophysical properties of the fluorophore (including photostability, quantum yield, Stokes shift, and fluorescence lifetime), various fluorescence-based detection strategies have been constructed. Given the advantages over organic fluorophores, different kinds of fluorescent nanoparticles have been designed and constructed. More significantly, combined with the development of DNA technologies, the construction of fluorescent DNA nanostructures has exhibited great advantages for biological applications, which largely required the precise design of DNA nanostructures.

DESIGN AND ASSEMBLY OF DNA NANOSTRUCTURES

The design of DNA nanostructures originated in the early 1980s,[36] then Seeman proposed a holliday junction of a mobile branched nucleic acid structure that joined with four double-stranded arms (Figure 2A).[37] Thereby, various DNA structural units such as DNA tiles lattice (Figure 2B),[38] DNA bricks (Figure 2C-left), scaffold origami (Figure 2C-middle), the single-stranded origami (Figure 2C-right) were created,[39] and sundry DNA nanostructures were emerged in succession.[40-42]

Especially in 2004, a nanoscale octahedron was generated by folding a 1669-nucleotide single-stranded DNA (ssDNA) in the presence of five 40-mer oligodeoxynucleotides through a simple denaturation–renaturation procedure.[43] This work is quite important, which has evoked the foundation of the epoch-making DNA origami technique, also called the preorigami technique. Thereafter, Paul Rothemund developed the DNA origami technology in 2006,[14] which has become an important milestone in DNA nanotechnology. With quite a lot of outstanding advantages, DNA origami has become an indispensable member of DNA nanostructures.[44] Typically, the shape of DNA origami is well-defined and can be easily designed with the help of various software. This top-down origami approach is a simple yet powerful technique in the field of DNA nanotechnology. Initiated by origami techniques, various DNA structures have been elaborately fabricated, including DNA nanonetworks (Figure 2D),[45] DNA nanocapsules (Figure 2E),[46] DNA nanorobots (Figure 2F),[47] non-periodic 2D structures,[48] 3D DNA structures (octahedron structures, tetrahedra DNA structures, etc.),[42,49,50] structures with complex curvatures,[51] and dynamic DNA structures.[52-54] Besides, DNA origami nanostructures also hold myriad possibilities for further modification due to their particular addressability. Consequently, the DNA nanostructures can be straightly decorated by multiple functional entities, such as fluorescent dyes, metal nanoparticles, enzymes, and functional nucleic acids. This would ensure the great performance of DNA nanostructures in biomedical applications.[55-61]

THE BIOMEDICAL APPLICATIONS OF FDNs

Upon exploring design principles and assembly methods for building complex DNA structures, DNA nanotechnology has reached an unprecedented level, facilitating biological applications of DNA nanostructures. In the past decade, numerous attempts at the construction of DNA-nanotechnology-based platforms have been employed for diagnosis and therapeutic applications. Due to their unique properties and diverse functionalities, FDNs have emerged as promising tools in biomedical applications. Functional modules can be feasibly integrated into FDN-based nanocarriers, allowing for real-time tracking, precise targeting, conditional activation, controlled release, and therapeutic agent delivery.[62] In addition, different fluorescent strategies have been integrated into the construction of functional DNA nanostructures, which has greatly enhanced the performance of detection, diagnosis or treatments. Herein, different fluorescent biomedical applications in DNA constructions have been reviewed, including DNA nanostructure-based FRET, NAT, AIE, NIR-photoactivation, nucleic acid amplification (NAT), small fluorescent dyes loading, and SPR technologies.

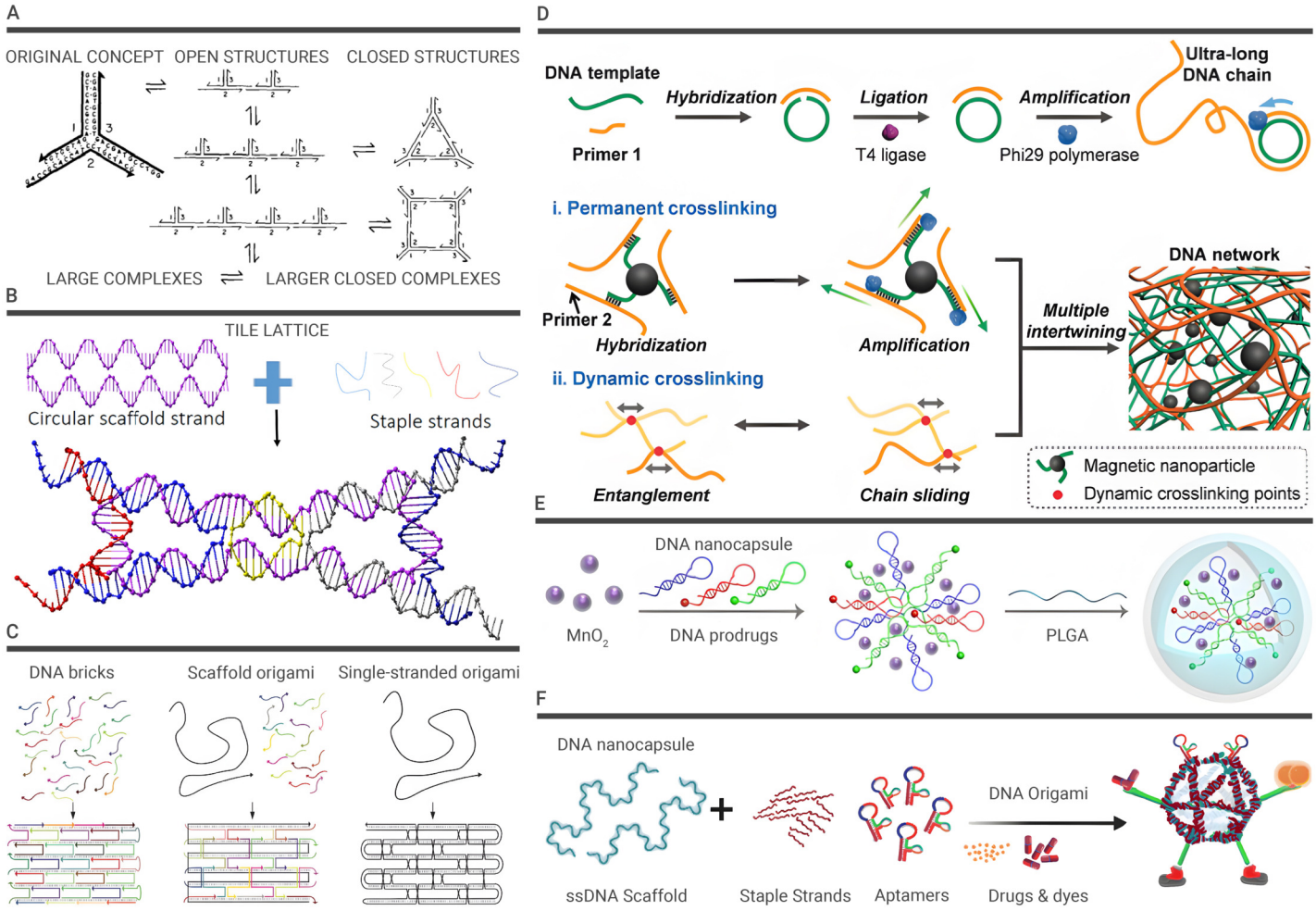

Figure 2. The design and classification of DNA nanostructures (A) The ligation of nucleic acid junctions.[37] Copyright © 1986, © IRL Press Limited, Oxford, England. (B) Self-assembly of the circular scaffold with the central core strand (yellow) and the staple strands to yield DNA tiles.[38] Copyright © 2022 Elsevier B.V. (C) DNA self-assembly of DNA bricks (left), scaffold origami (middle), and single-stranded origami (right).[39] Copyright © American Association for the Advancement of Science. (D) Synthesis route of the magnetic DNA nanonetwork.[45] Copyright © 2019 Wiley-VCH Verlag GmbH.(E) The preparation of multifunctional nanocapsule.[46] Copyright © 2020 Wiley-VCH GmbH. (F) Fabrication of DNA nano/microrobots in modern theranostics.[47] Copyright © 2024 Copyright Clearance Center, Inc.

Fluorescence resonance energy transfer (FRET)-based DNA nanostructures

FRET normally occurs once the donor is close to the acceptor (a few nanometers), in which an excited donor chromophore transfers energy to an acceptor chromophore via nonradiative dipole–dipole coupling. Based on changes of fluorescence signals during FRET, the FRET-based nanoparticles have been constructed for the diagnosis.[63–65] In addition, owing to the capability to provide real-time spatial measurements between the donor and acceptor fluorophores, the FRET-based nanomaterials are powerful for the sensitive detection of cancer biomarkers even for *in vivo* imaging. The efficiency of FRET-based nanomaterials plays a crucial role in diagnosis performance, which is dependent upon several factors: (1) the spectral overlap between absorbance of the acceptor fluorophore and emission of the donor fluorophore (Figure 1A), (2) their relative orientations, and (3) their proximity (because the fluorescent efficiency is inversely proportional to the distance between the acceptor and the donor).[64] The examinations of these factors have effectively guided the design of the FRET-based nanomaterials for biological applications. Given fluorophores with a large Stokes shift (typically larger than 80 nm, while 55 to 80 nm is acceptable)[66,67] can contribute to avoid self-quenching and light scattering,[68,69] the sensitivity and accuracy of FRET-based biological detections can be increased.[70,71] For example, a FRET-based platform was fabricated with two fluorophores with both large Stokes shifts as donor and acceptor (Figure 3A). As examined, their maximum excitation/emission wavelengths were 475/535 nm and 545/735 nm, and the Stokes shifts were 60 nm and 190 nm. Thus, upon encapsulating in the same micelle, FRET effect was occurred to assess the integrity of polyethylene glycol-block-poly(ε-caprolactone) micelles *in vivo*.[72]

Especially, FRET is most commonly introduced into the construction of switch fluorescent DNA nanostructures to enable FRET-based biological applications. The unique merits of DNA play an important role in realizing the fluorescence "off-on" switching of fluorescence. Firstly, DNA nanotechnology provides a versatile platform for the energy transfer upon precisely controlling the distance and orientation between donor and acceptor molecules. For instance, DNA origami structures acted as scaffold templates to position fluorophores with nanometer-scale precision, allowing for efficient energy transfer.[14] Additionally, DNA's dynamic and tunable conformational properties enabled designing switchable FRET systems, where the responses would be altered upon DNA conformational changes by target binding.[73] Furthermore, DNA's compatibility with gold substrates through Au-S chemistry also enabled the integration of plasmonic nanoparticles to enhance FRET efficiency and sensitivity.[74] Consequently, by combing DNA nanotechnology with FRET-based detections, biomolecules can be sensitively detected based on fluorescent changes induced by a conformational change of FDNs.

For the biological applications, folding-upon-repair DNA nanoswitches containing O6-MeG nucleobases have been designed, which underwent a conformational switch from duplex to triplex upon enzymatic demethylation (Figure 3B).[75] Recognized by the methyltransferase enzymes, the methylated nanoswitches efficiently led to the measurable FRET signal changes, which facelifted the evaluation of the conformational switch. In addition, a DNA tetrahedron-based nanoswitch probe (TD) was developed for ratiometric

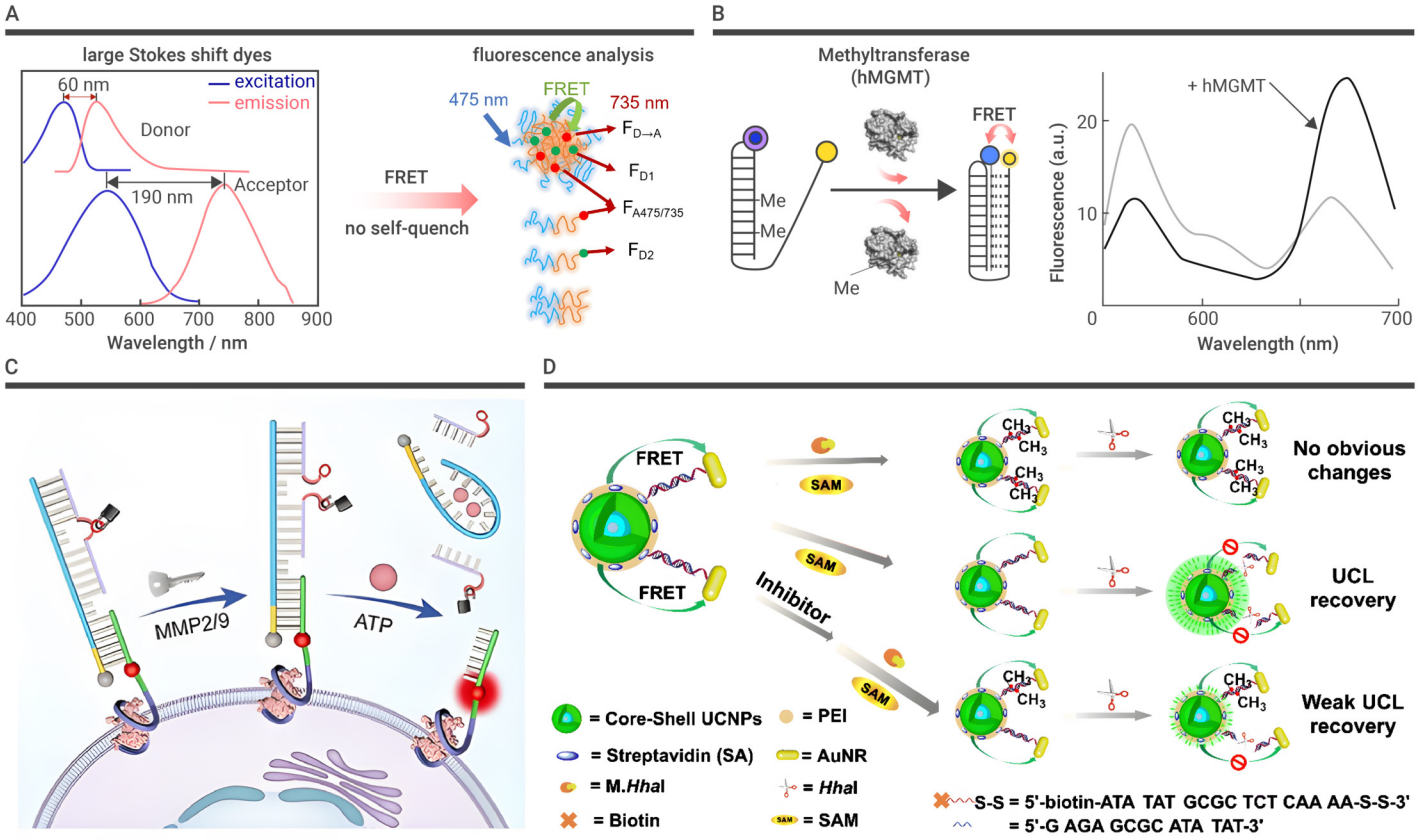

Figure 3. FRET-based DNA nanostructures for biomedical application (A) Excitation and emission spectra of the donor and acceptor (left) and the large Stokes shift fluorophore-based FRET (right).[72] Copyright © 2020 Elsevier B.V. (B) Folding-upon-repair DNA nanoswitches for monitoring the activity of DNA repair enzymes.[75] Copyright © 2020 Wiley-VCH GmbH. (C) The engineering of multivariate-gated DNA nanodevice for spatially selective, correlated imaging of MMP2/9 and ATP in extracellular TME.[82] Reprinted with permission from 2021 Wiley-VCH GmbH. (D) Detection procedures of M.HhaI MTase by FRET probe based on UCNPs and AuNRs.[106] Copyright © 2021 Springer Nature.

fluorescence imaging,[76] by hybridizing two split ATP aptamers to a DNA tetrahedron. As designed, the presence of ATP will change the structure of TD from the open state to the closed one, which emitted high FRET signals upon decreasing the distance of dual fluorophores. Besides, the functional DNAs with structure-switching activities can be designed to act as anti-ATP aptamers,[77,78] exhibiting immense promise in engineering probes for ATP sensing and imaging.[79-81] For instance, a DNA nanomachine was reported for ATP imaging in tumor,[79] with a structure-switching aptamer as the ATP sensing module (Apt).[78] The Apt module was constructed by hybridizing a Cy5-labeled aptamer strand with a quencher (BHQ2)-bearing complementary strand, whose fluorescence was quenched by the FRET. After intravenous injection, the DNA nanomachine could be specifically anchored on tumor cells and the fluorescence was displayed with a high signal-to-background ratio. Furthermore, a multivariate-gated DNA nanodevice (At-MAs) was designed for enantioselective AND-gate imaging of MMP2/9 and ATP in extracellular TME.[82] As shown in Figure 3C, a DNA nanodevice was constructed by modifying a MMP2/9-hydrolyzable PNA/peptide copolymer with cell membrane-anchoring aptamer and an ATP-responsive aptamer. Therefore, with 4T1 xenograft as a highly aggressive tumor model, the robustness of the DNA nanodevice in enantioselective imaging of MMP2/9 and ATP in TME was confirmed. In addition, the FDN-based nanoswitch can also be used for sensitive detection of Dam MTase activity,[83] monitoring the anchor of FDN nanodevices and in situ self-assembly on target living cell surfaces.[84] Therefore, the FRET-based FDNs have exhibited great advantages for sensitive detection and evaluation of biological molecules.

Except for detections, FRET-based artificial DNA nanomachines can also guide their movements along DNA footpaths[85-87] including two-dimensional DNA origami tracks,[88,89] or three-dimensional tracks.[90-92] In particular, DNA machines with three-dimensional DNA-Au nanoparticle tracks[93-101] have attracted great interest, which integrated the thiolated oligonucleotides onto a single AuNP surface via Au-S bonds. For example, propelled by exonuclease III, a stochastic DNA walker was designed to move on an AuNP-based 3D track autonomously. For detections, a 6-carboxyfluorescein (6-FAM) was tagged at the 3'-end of DNA to act as the signal reporter.[90] Besides, a bipedal DNA walker was developed, which conjugated hundreds of fluorescent single-stranded DNA (ssDNA) substrates to AuNPs through the well-established Au-S chemistry.[100] Furthermore, with nuclease- or peroxidase-mimicking catalytic DNA sequences, DNAzymes can be devoted to the cleavage of RNA or DNA in the presence of metal ions. Therefore, with their potential effective driving force, DNAzymes have been regarded as ideal candidates for designing a DNA nanomachine.[102-104] For example, with a long DNAzyme motor strand conjugated to the AuNPs, a DNAzyme motor was developed for moving on the AuNP surface stochastically.[105] The whole DNA tracks contained hundreds of substrate strands and dozens of DNAzyme molecules that silenced by a locking strand. Consequently, upon intracellular interaction with a target molecule, the DNA motor system was initiated for the autonomous walking on the AuNP. Simultaneously, the quenched fluorescence of FAM by AuNP was turned on through FRET, which facilitated the real-time imaging of the motor behaviors in living cells. Similarly, a fluorescence transducer based on FRET between the acceptor gold nanorods (AuNRs) and the donor upconversion nanoparticles (UCNPs) is constructed with a specific ssDNA as the linker.[106] As shown in Figure 3D, the fluorescence intensity of UCNPs presented excellent photostability over 60 min and was hardly affected by changes of temperature and pH, which exhibited excellent performance to evaluate MTase activity. More interestingly, without DNAzyme strands conjugated to AuNPs as the DNAzyme motor did, a DNAzyme walker[107-110] could processively and autonomously move on the AuNPs. In this way, Chu's group reported a single DNAzyme-based walker for *in vitro* real-time monitoring of the machine operation as well as the imaging of intracellular miRNA-21.[110] To further enhance the walking, they designed a photo-controlled and self-powered bipedal DNA walking machine,[111] for real-time monitoring of the intracellular miRNA. Powered by substrate cleavage,

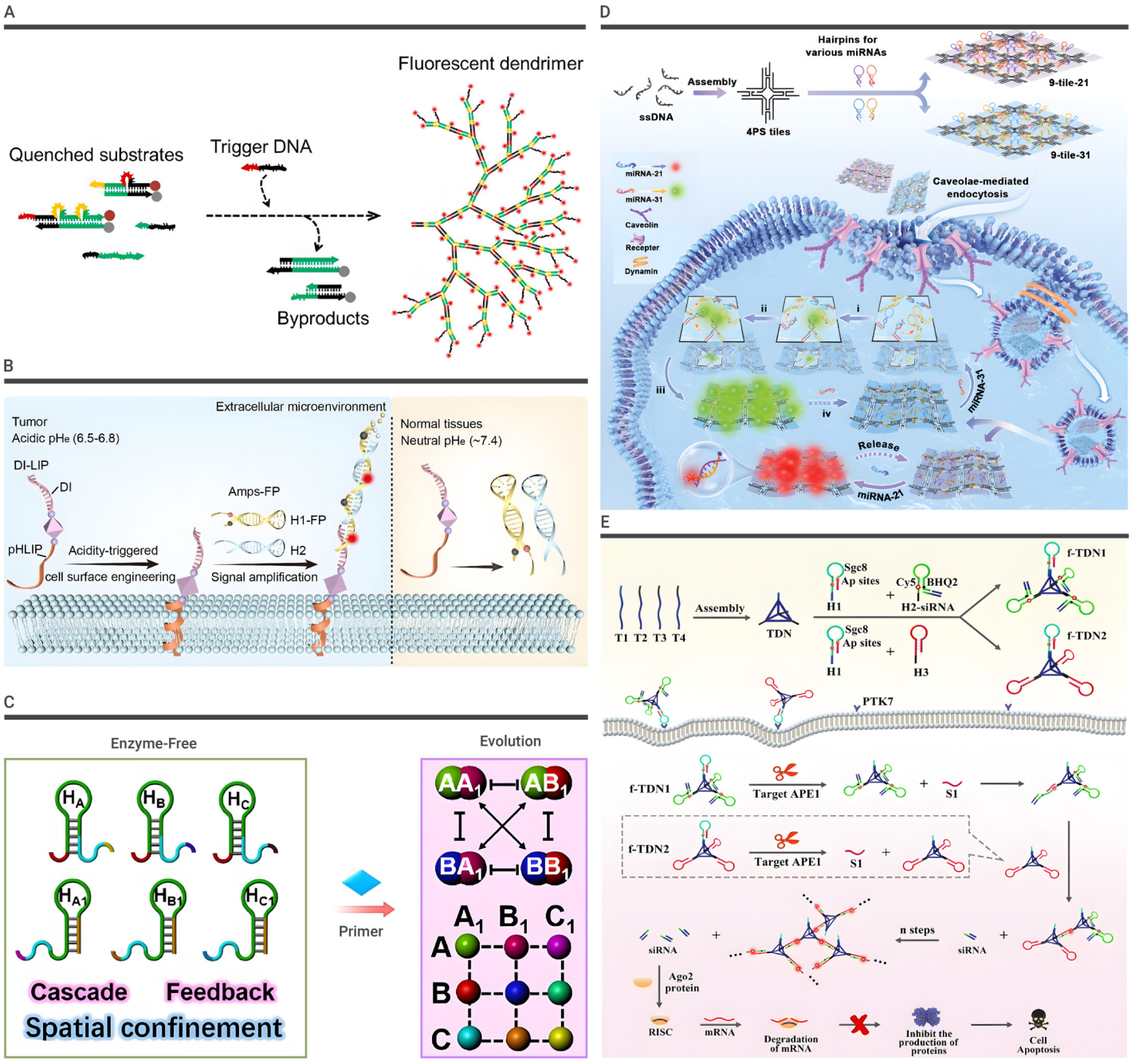

Figure 4. Nucleic acid amplification-based DNA nanostructures (A) Components and reaction pathway for the triggered chain-branching growth of fluorescent DNA dendrimers.[126] Copyright © 2014 American Chemical Society. (B) The principle of MAT-amp for spatially selective signal amplification of mild acidosis in TME via pH (low) insertion peptide-mediated recruitment of DNA amplifiers.[127] Copyright © 2022 Wiley-VCH GmbH. (C) Cascaded, feedback-driven, and spatially localized emergence of constitutional dynamic networks driven by enzyme-free catalytic DNA circuits.[130] Copyright © 2023 American Chemical Society. (D) Schematic diagram of caveolae-mediated internalization of the 9-tile nanoarrays for amplified imaging of miRNAs in living cells.[129] Copyright © 2023 Wiley-VCH GmbH. (E) The assembly of DNA structures, the principle of sensitive detection and fluorescence imaging of APE1, and apoptosis of tumor cells.[131] Copyright © 2024 Copyright Clearance Center, Inc.

this bipedal DNA walking machine exhibited enhanced sensitivity with a lower detection limit of 3.51 pM. Thereby, upon the labeling of substrate strands with FAM, real-time monitoring was enabled in living cells.

Furthermore, single-molecule FRET (smFRET) technology has been proposed for real-time tracking of changes in a single molecule or molecular complex at the nanoscale. The smFRET technique has the capability of receiving real-time structural and functional information on these DNA-made nanostructures. At present, DNA nanostructure-based smFRET has become an ideal technique to study the structure and dynamics of proteins and nucleic acids. These include observing the conformational changes of the enzyme during its functional cycle,[112] real-time monitoring of DNA synthesis,[113] and understanding the spatial distribution of target molecules in cells.

With the development of programmable DNA technology and the use of next-generation sequencing methods, the design and construction of DNA nanostructures have been greatly promoted. These DNA nanostructures can be precisely tailored to provide a rigid and defined platform for FRET experiments. Consequently, controlling the distance and orientation between donor and acceptor of fluorophores, developments of DNA nanostructures have opened up new opportunities for FRET-based sensing and imaging. Nevertheless, although FRET-based detections have promoted developments of FDNs for biological applications, other issues such as detection of ultra-trace biological species are still encouraged.

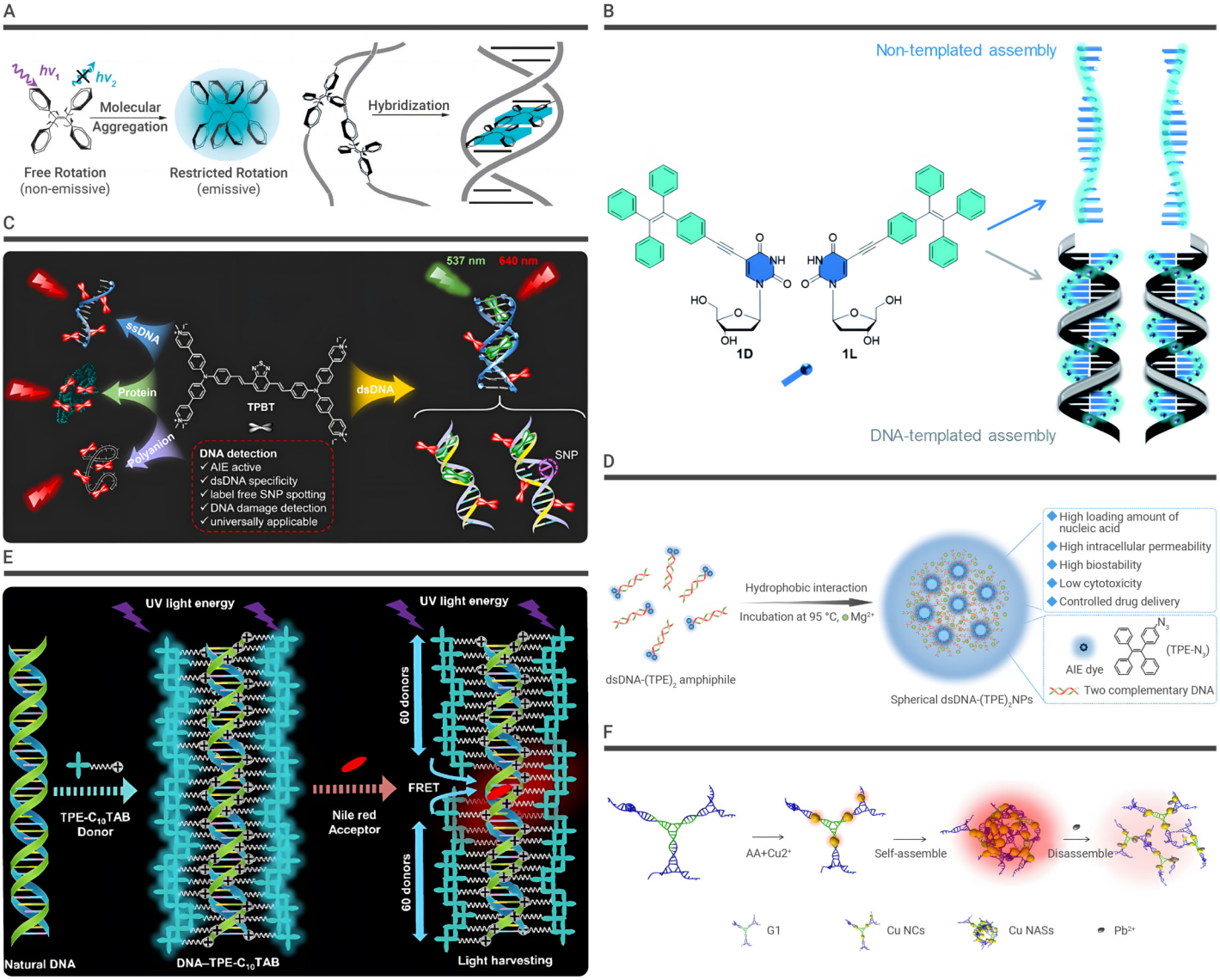

Figure 5. AIE properties and AIE-based DNA nanostructures' applications (A) AIE properties of tetraphenylethylene (a) as a monomer and (b) as a building block in single and double-stranded DNA.[155] Copyright © 2012 American Chemical Society. (B) Illustration of the non-templated assembly and DNA-templated assembly of tetraphenylethylene-modified 2'-deoxyuridines 1d and 1L.[156] Copyright © 2024 Copyright Clearance Center, Inc. (C) Molecular structure of TPBT and its various emissions upon binding with different polyanionic macromolecules.[158] Copyright © 2019 American Chemical Society. (D) The formation of spherical dsDNA-$(TPE)_2$ NPs from two complementary DNA conjugated with hydrophobic TPE molecules due to hydrophobic interaction.[160] Copyright © 2022 Wiley-VCH GmbH. (E) Schematic illustration of dense binding of AIE donors into natural DNA for constructing an efficient light-harvesting system.[161] Copyright © 2023 Wiley-VCH GmbH. (F) Schematic diagram of the preparation of DNA dendrimer-Cu NASs and Pb^{2+} sensing.[162] Copyright © 2021 Wiley-VCH GmbH.

Nucleic acid amplification technology (NAT)-based DNA nanostructures

Direct *in situ* analysis of intracellular nucleic acid has provided valuable approaches for identifying cancerous cells and monitoring changes in nucleic acid levels.[114-118] However, visualization of intracellular cancer-associated DNA/RNA remains a key challenge due to its low expression levels and inadequate signal readout.[119-121] Consequently, it is imperative to develop intracellular signal amplification strategies for monitoring cellular DNA/RNA in ultralow amounts.[61,122] Nucleic acid amplification allows detection of low-abundance targets at an ultrahigh amplification efficiency (over 10^8), upon specifically identifying genetic markers by the designed primers. Especially, DNA nanostructures can be designed as scaffolds for the assembly of multiple amplification components, such as primers, enzymes, and target-specific probes. This integration of amplification components into DNA nanostructures enables efficient signal amplification, reducing false-positive results and improving detection limits. Additionally, the programmability of DNA nanostructures allows for multiplexed amplifications and detections, enabling simultaneous analysis of multiple targets.[123] Therefore, the introduction of nucleic acid amplification into DNA nanotechnology significantly enhances the sensitive and specific detections of biological molecules.

For introducing amplification strategies into constructing DNA nanostructures, Pierce and co-workers demonstrated a dynamic assembly system to form dendritic nanostructures by triggering DNA hairpins.[124] Recently, a nonlinear version of hybridization chain reaction (HCR) was reported to construct an exponentially growing dendritic nanostructure by two-loop structured hairpins.[125] In addition, a nonlinear HCR system was also introduced to achieve exponential growth kinetics, in which a trigger DNA initiated the self-sustained assembly of quenched double-stranded substrates into fluorescent dendritic nanostructures. As shown in Figure 4A, upon strand-displacement reactions, multiple trigger DNAs labeled with fluorescent reporters were released from quenched substrates.[126] This facilitated the chain-branching growth of the assembled DNA dendrimers along with a rapid increase in fluorescence intensities. Similarly, a DNA-based strategy was also reported to enhance the detection sensitivity of acidic signals in the extracellular environment of tumors (Figure 4B).[127] Therefore, NAT-based fluorescent DNA nanostructure technology can detect trace biological targets in cells, which is of great significance for the early screening of tumors.

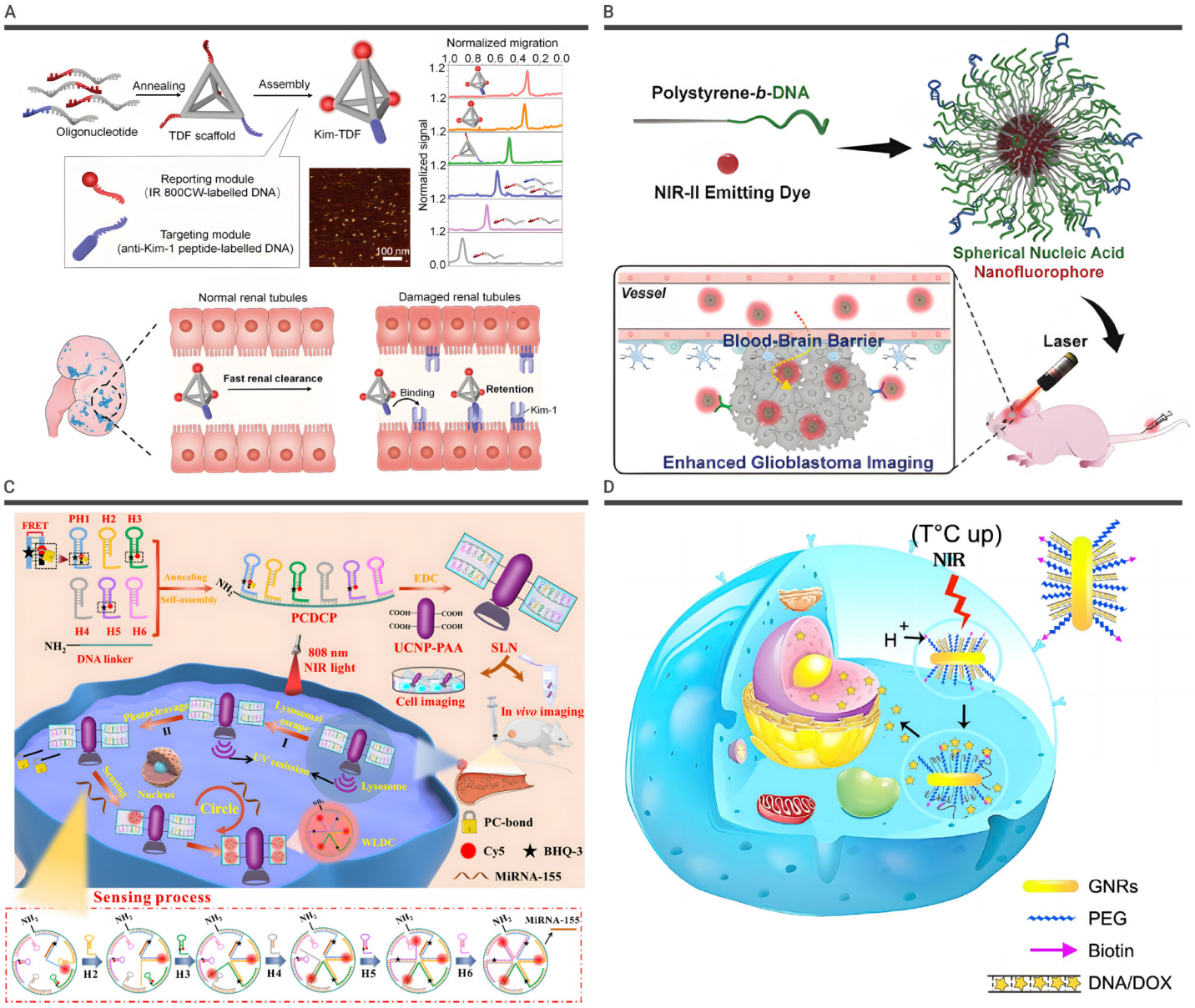

Figure 6. The application of NIR-based DNA nanostructures (A) Design and characterization of the engineering TDF nanodevice (Kim-TDF).[171] Copyright © 2022 Wiley-VCH GmbH. (B) Illustration of organic fluorescence-emitting spherical nucleic acid self-assembled from PS-b-DNA and organic dyes.[172] Copyright © 2020 Wiley-VCH GmbH. (C) Establishment flow of SLN and operation principle of the SLN-supported fluorescent nanosensor in live biological medium.[176] Copyright © 2023, American Chemical Society. (D) pH and near-infrared light dual-stimuli responsive drug delivery using DNA-conjugated gold nanorods for effective treatment of multidrug resistant cancer cells.[173] Copyright © 2016 Published by Elsevier B.V.

In addition, the enzyme-free catalytic hairpin assembly (CHA) also acted as a guided and high-throughput reaction module for the evolution of constitutional dynamic networks from a set of nucleic acids. In this way, the self-assembly nanoarrays of micrometer-scale DNA tiles have been constructed upon the efficient CHA. Upon spatially restricted CHA with accelerated kinetics, the ultrasensitive detection of miRNA-21 has been achieved *in vitro*.[128] Afterwards, the self-assembly of DNA tiles was modulated (Figure 4D)[129] and size-controlled nanoarrays were constructed in the nanoscale. With this platform, direct cytoplasmic delivery via caveolae-mediated endocytosis was achieved, and the signal-amplified dual-fluorescence imaging of miRNAs was realized at the single-cell level. Recently, networks of variable complexities and functionalities have been assembled into constitutional confinement dynamic networks (CDN) by CHA, which provided possible pathways for examining evolutions under prebiotic conditions. In this way, a set of four or six structurally engineered hairpins was subjected to a promoter P1, leading to the CHA-guided emergence of a [2×2] CDN or the evolution of a [3×3] CDN, respectively (Figure 4C).[130] Besides, to enhance the targeting activity and reaction efficiency, the DNA networks were assembled from two kinds of functionalized tetrahedral DNA nanostructure (Figure 4E).[131] As demonstrated, the reaction rate of the CHA on functionalized tetrahedral DNA nanostructures was much faster than that of the conventional free CHA reactions. This could be attributed to the high local concentration of hairpins, spatial confinement effect, and production of giant DNA networks, which significantly enhanced the fluorescence signals.

In addition to the conventional nucleic acid amplification strategies of HCR and CHA, there are some other strategies to trigger signal aggregations. For example, combined with FRET strategy, the performance of FDNs has been further enhanced. In this way, the AuNPs-based amplified FRET nanoflare was constructed, which provided significantly amplified FRET signals from multiple signal strands on AuNPs. As designed, once triggered by miRNA, high-abundance endogenous mRNAs would act as fuels and generate amplified FRET signals for the imaging of low-abundant targets in living cells.[132] For the more biocompatible sensitive detections, a programmable AuNPs-based DNA nanoflare was further established upon the encapsulation by

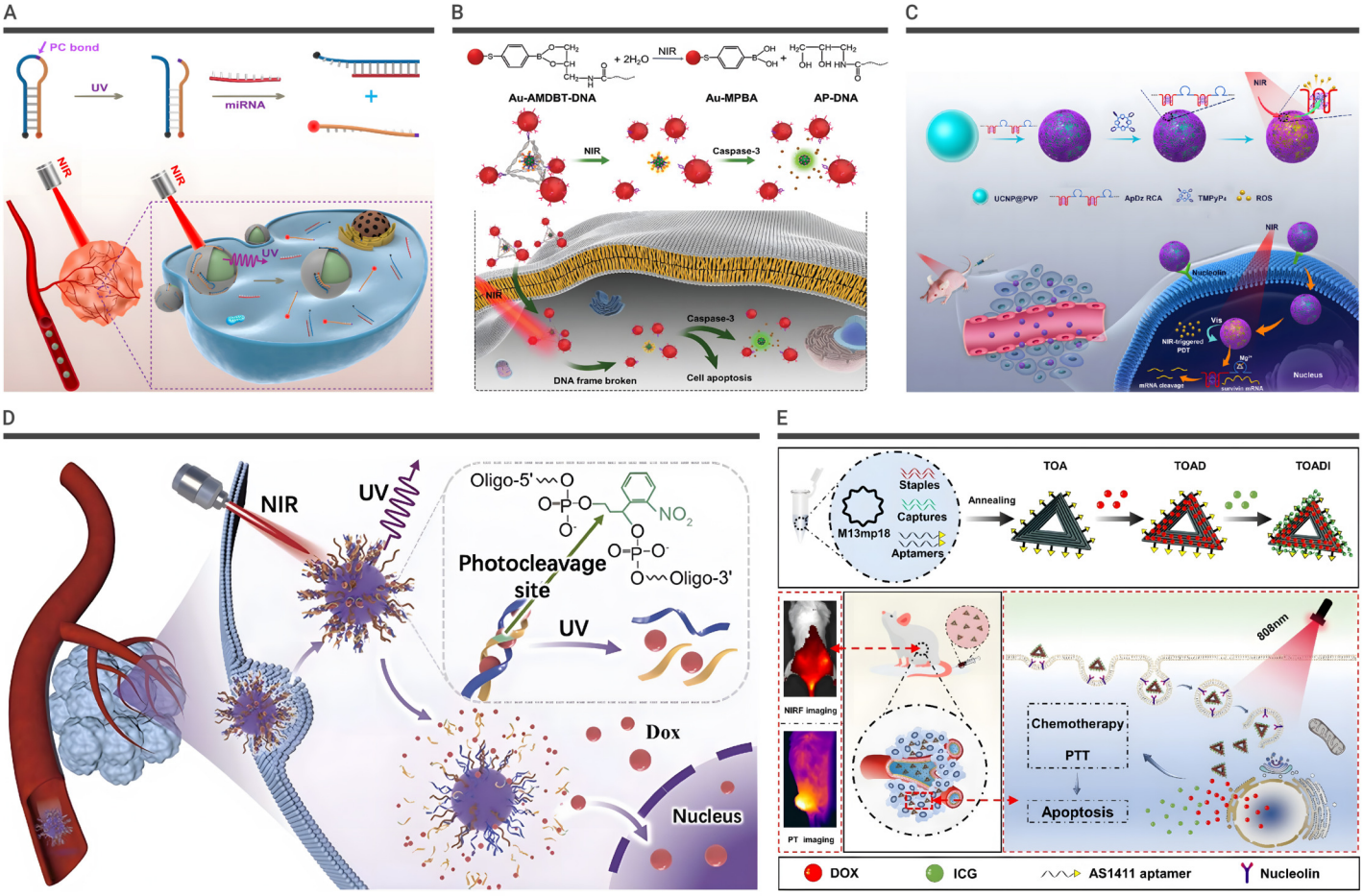

Figure 7. The application of UCNPs-based DNA nanostructures in cancer treatments (A) Working principle of the activatable DNA nanodevice for NIR light controlled miRNA sensing and imaging.[181] Copyright © 2019 American Chemical Society. (B) Schematic illustration of NIR-induced disassembly of UAuTe tetrahedron and the use for senescence clearance and apoptosis tracking *in vivo*.[183] Copyright © 2020 Wiley-VCH GmbH. (C) Illustration of the synthesis of multifunctional DNA polymer-assisted upconversion therapeutic nanoplatform and the targeted photodynamic nanoplatform for highly efficient photodynamic therapy.[184] Copyright © 2020 American Chemical Society. (D) Schematic illustration of the working mechanism of the NIR light-responsive DNA nanodevice.[185] Copyright © 2022 Elsevier Ltd. All rights reserved. (E) Schematic illustration showing the DNA origami-based nanoplatforms for synergistic cancer therapy involving PTT and chemotherapy against breast cancer *in vitro* and *in vivo*.[186] Copyright © 2023 BioMed Central.

cancer cell membrane vesicle.[133] In this work, a poly(A)-tagged and FAM-labeled hairpin DNA probe was modified on the surface of the AuNPs, and the FAM fluorescence was quenched by the AuNPs via FRET. After homing-targeting internalization, the specific miRNAs triggered the fast dynamic catalytic hairpin assembly to display an "off-on" fluorescence response, which facilitated the sensitive intracellular miRNA imaging. Furthermore, a rationally designed elegant entropy-driven DNA probe was integrated with assisted DNA fuel on hollow copper sulfide nanoparticles (HCuSNPs).[134] The anchored assisted DNA fuel strands could be efficiently released upon photothermal effect of the HCuSNPs under a NIR-II laser irradiation. The DNA machine was activated by the target binding of miRNA and was powered by the released DNA fuels through toehold-mediated strand displacement reactions. In another work, a dynamic DNA origami plasmonic nanodevice aided by a DNA circuit was reported to convert trace stimuli signals to the detectable NIR CD responses.[135] Furthermore, an *in vivo* miRNA magnifier programmed by 980/808 nm NIR was developed by conjugating an activatable DNAzyme walker to rare-earth nanoparticles.[136] As designed, the 980-nm excitation has activated DNA nanomachine for miRNA-21 recognition, followed by signal amplification to suppress "false positive" signals.

Consequently, by integrating nucleic acid amplification techniques with fluorescent DNA nanostructures, highly sensitive and specific detection of target biomarkers were achieved. These nanostructures can be designed to recognize specific DNA or RNA sequences and generate amplified fluorescence signals upon target recognition. Combined with multiple fluorescent strategies, the detection of infectious agents, genetic mutations, or disease-related biomarkers with high accuracy and sensitivity would be greatly facilitated.

Aggregation-induced emission (AIE)-based DNA nanostructures

Expect for the enhancement of fluorescent signals by amplification techniques, some other strategies could also provide enhanced fluorescent signals for the sensitive detections. Especially, AIE has provided enhanced responses for sensitive detections, which is a photophysical phenomenon closely related to the intramolecular motion of excited states (Figure 5A).[137] Normally, the sensitivity and photostability of small fluorescent molecules are limited by the aggregation caused quenching (ACQ) effect and consequently weakens their performance in biosensing applications.[138,139] In contrast to the traditional ACQ effect, AIE luminogens (AIEgens) display much higher fluorescence and possess advantages for biological applications (such as low background, long-term tracking ability, and strong resistance to photobleaching). In the dispersed states, dynamic intramolecular rotation and vibration of AIEgens give rise to the nonradiative decay and dissipate the excitation energy. While under aggregation, strong fluorescence is exhibited upon intramolecular motion[140] of molecules, preventing the excited-state energy dissipation and initiating a barrier to nonradiative decay. In this pathway, AIEgen molecules have been designed for detecting ions,[141] amino acids,[142] carbohydrates,[143] nucleic acid[144] and peptides/proteins.[145] Recently, amphiphilic AIE polymers have emerged as highly attractive luminogens, upon the construction of functional polymeric self-assemblies with synergistic effects.[137,146] Therefore, AIEgens have also been involved in

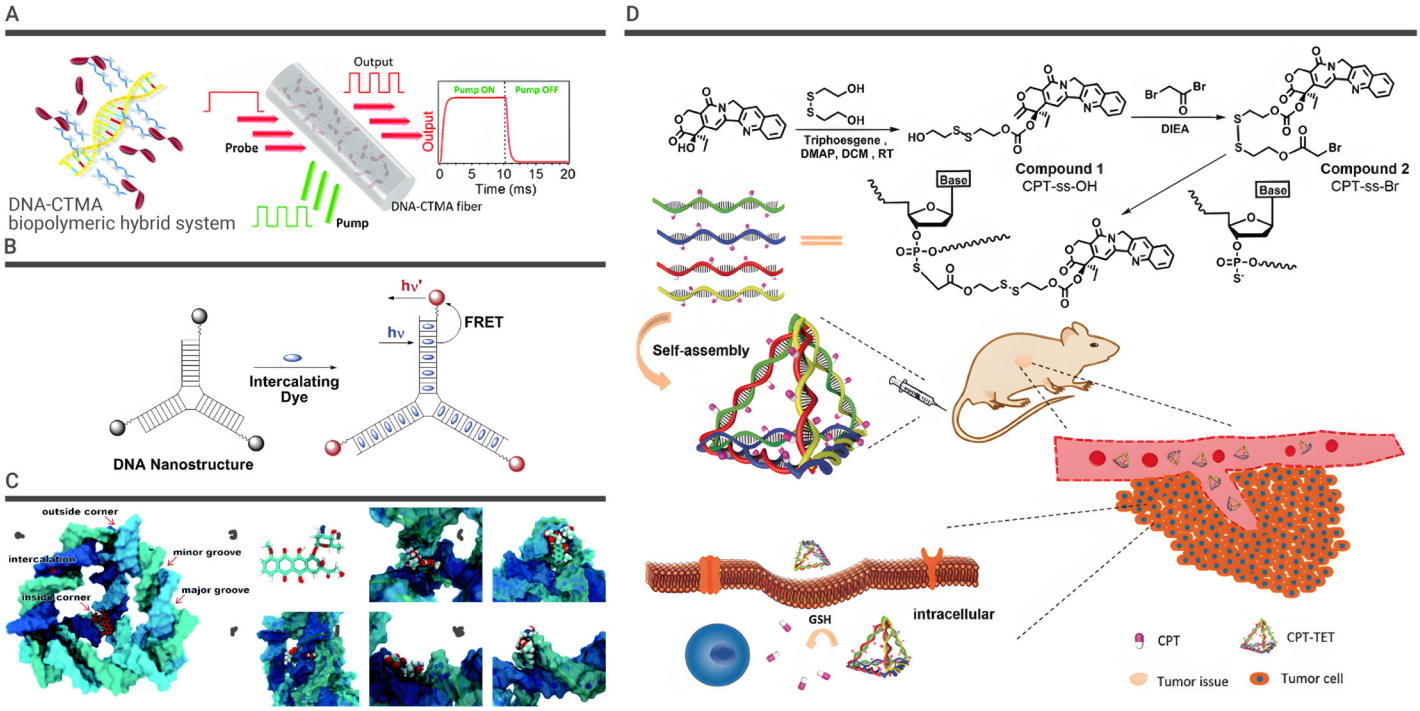

Figure 8. Small fluorescent dye-intercalated DNA complexes (A) Scheme of the photoisomerizable dye semi-intercalation in the DNA-CTMA matrix and all-optical switching in dye-doped DNA nanofibers.[188] Copyright © 2024 Copyright Clearance Center, Inc. (B) Self-quenching is avoided by confining the dyes within separate intercalation sites.[192] Copyright © 2007 American Chemical Society. (C) Schematic illustration for a single DOX binding to the TDN-17 and (b) the structural formula of the DOX. The possible binding modes observed in the molecular docking: (c) the inside-corner mode, (d) the outside-corner mode, (e) the intercalation mode, (f) the major-groove mode, and (g) the minor-groove mode.[193] Copyright © 2024 Copyright Clearance Center, Inc. (D) The synthetic routes of CPT-modified phosphorothioate oligonucleotides and their self-assembly to form CPT-loaded DNA tetrahedra, as well as the delivery process.[195] Copyright © 2019 Wiley-VCH GmbH.

the construction of photostable biosensing nanodevices for biological applications.

To promote the biological application of AIE-based strategies, more and more reports have been developed for describing the interaction of AIE molecules (AIEs) with DNA,[147,148] proteins[149,150] or other biostructures.[151] Naturally, DNA nanotechnology offers unique contributions to AIE-based detections. Typical, DNA's hydrophobic pockets and hierarchically ordered structures can serve as excellent AIE-active molecules, preventing their aggregation in DNA nanostructures and thus maintaining fluorescence quenching.[152] Besides, the three-dimensional arrangement of DNA nanostructures further contributes to induce or enhance AIE effects upon the formation of chiral environments.[153] Therefore, by integrating AIE-active molecules into DNA nanostructures, effective fluorescence "on-off" switching can be achieved for biosensing. For example, an AIE compound of 1,1-dimethyl-2,5-bis[4-(isothiocyanatemethyl)phenyl]-3,4-diphenylsilole (SITC) was synthesized to be conjugated with aminoallyl-dUTP. As demonstrated, the quantum yields (Φ_F) was 45.8% in the solid state to guarantee the construction of AIE-based DNA systems. As designed, SITC showed weak emission in good solvents such as tetrahydrofuran (THF), while the PL intensity greatly increased with increasing water content. The PL responses in 95/5 (v/v) water-THF was approximately 50-fold higher than that in 70/30 (v/v) water-THF. With this platform, an AIE-based DNA nanostructure has been constructed upon the derivation of an AIE-active silole into DNA strands by an enzymatic method.[154] Furthermore, two stereoisomers of alkynyl-tetraphenylethylene were synthesized, respectively exhibiting Φ_F of 40% and 39% in a 95/5 (v/v) water-THF solution. The two stereoisomers were therefore incorporated into DNA to provide the conjugates with the AIE properties for sensitive biosensing.[155] In addition, two chiral conjugates of AIE dyes (tetraphenylethylene, TPE) (1D and 1L) were applied to constructing supramolecular DNA architectures with strong AIE properties upon self-assembly (Figure 5B).[156] The configurations of their 2'-deoxyribofuranosides are different, which can monitor the chirality of both the non-templated assembly and the assembly along *D*- and *L*-DNA templates. The modulating of the light-harvesting was achieved upon the efficient energy transport to the Attodye that attached to the 5'-terminus of the DNA templates. These supramolecular architectures were hierarchically ordered, whose DNA templates can modulate multiple functions including binding selectivity, chirality, and light harvesting capabilities.

More significantly, AIE-based FDNs have exhibited great potential in tumor-associated nucleic acid detections.[157] For example, upon binding to anionic macromolecules, the AIE luminogens (AIEgen) emitted bright red fluorescence emission at 640 nm. Based on this emission, a powerful molecule with AIE properties has been developed, to specifically interact with double-stranded DNA (dsDNA) via groove binding. With this platform, the conformational change of dsDNA can be detected based on AIE signal changes from red to green (Figure 5C).[158] In addition, the unique fluorescence of AIE was sensitively influenced by the base pairing of dsDNA, providing a new and facile approach for the label-free monitoring of SNP spotting and DNA damage. However, green emission near 537 nm was found to be an exclusive response of multinational AIEgen (called TPBT) to dsDNA. This signal was closely related to conformational changes after the groove binding of TPBT to dsDNA. Nobe et al. designed and synthesized block copolymers containing sulfonium cations and a hydrophobic TPE unit. This copolymer exhibited distinct self-assembly behaviors for generating polyplex with DNA, along with the AIE emissions with tunable colors for monitoring.[159] In addition, the emission related to charged states of dyes can be modulated by the formation of a polyplex between the negatively charged DNA and the positively charged sulfonium segments in the shell. These works have demonstrated the potential of AIE-based DNA nanostructures for biological applications.

With the developments of AIE techniques, the modification of AIE molecules has been employed to further enlarge the biological application of AIE-based DNA nanostructures. For example, a hydrophobic TPE was designed to be bound to an oligonucleotide,[160] which greatly enhanced the condensation properties of DNA (Figure 5D). As designed, two complementary DNA-TPE sequences were close for the hybrid (dsDNA-$(TPE)_2$) in an Mg^{2+}-containing buffer, which induced the self-assembly for obtaining spherical nanostructures. Besides, based on the fluorescent properties of AIE molecules, the aggregation state can be indicated by the sensitive fluores-

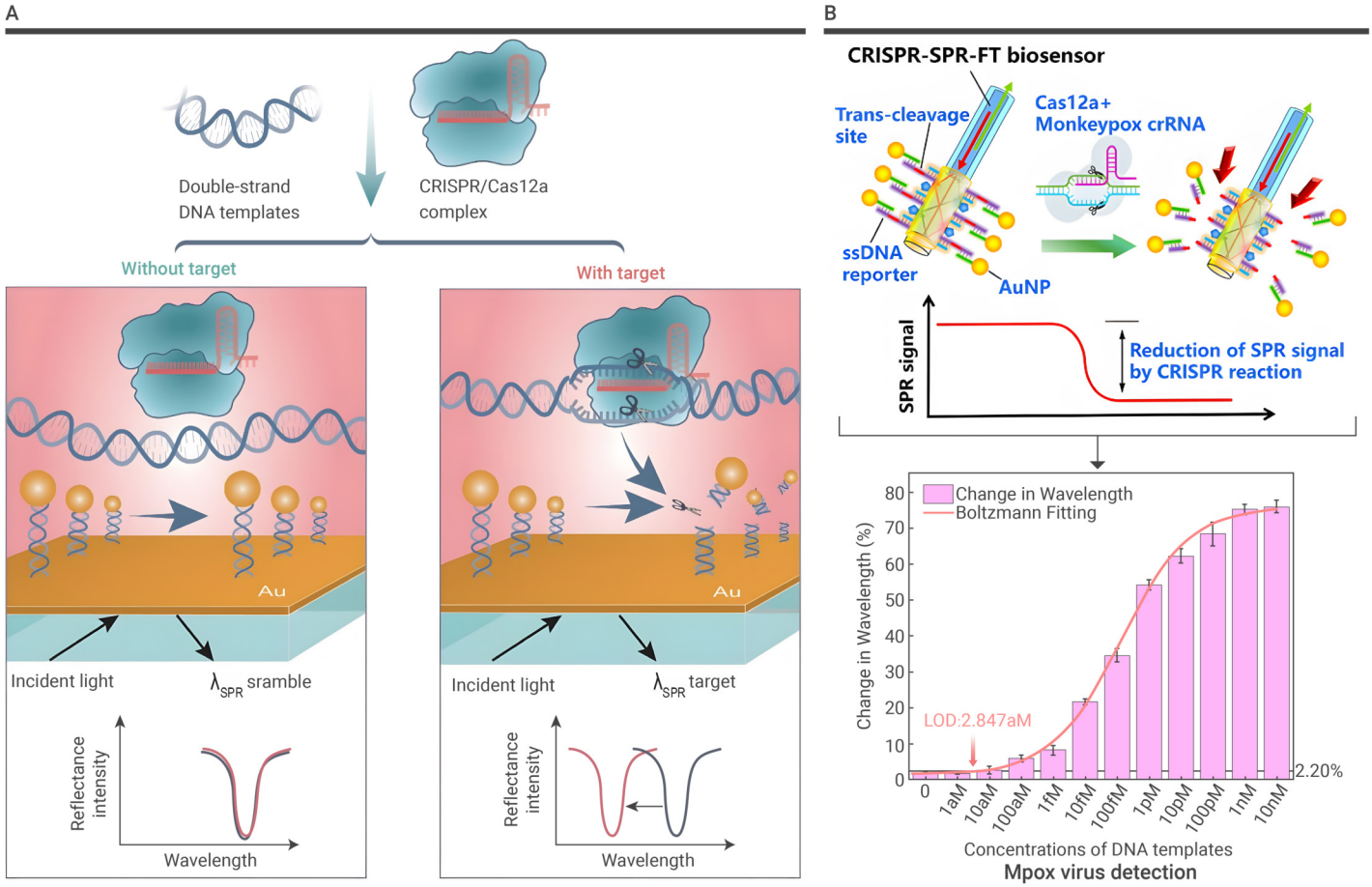

Figure 9. The detection methods with SPR-based DNA nanostructures (A) Methodologies of a photonic CRISPR sensing to induce a changed (decreased) SPR wavelength.[202] Copyright © 2022 Oxford University Press. (B) The design of the portable CRISPR-SPR-FT biosensing platform for MPXV detection.[203] Copyright © 2023 American Chemical Society.

cence. This not only facilitated studying the self-assembly mechanism of AIE but also being conducive to monitoring the cellular processes during gene transfection. As demonstrated, the hybrid dsDNAs showed significant fluorescence upon TPE aggregation, which was about tenfold higher than the sum signals from multiple units of single DNA-TPE. Moreover, to obtain an efficient light-harvesting antenna upon the assembly of dense donors around a single acceptor, a cationic AIE amphiphile was further designed to self-assemble with natural DNA duplexes.[161] As illustrated in Figure 5E, the as-prepared donor of cationic AIE amphiphile was densely attached to the phosphate groups of natural DNA duplexes through smaller cationic trimethylammonium. In this process, the insufficient binding caused by the steric hindrance of the AIE fluorophore can be overcome by the long alkyl chain between the cationic trimethylammonium and AIE fluorophores. This ensured a remarkably high donor/acceptor ratio for biological applications, which was comparable to that of the custom DNA assemblies.

Besides, AIE-based DNA nanostructures can also be used for the detection of metal ions. For instance, Cu^{2+} can be detected by DNA-copper nanoassemblies with AIE enhancement properties, which were designed using DNA dendrimers with the sticky ends as templates (Figure 5F).[162] In addition, the FDNs can also act as an excellent fluorescent probe for detecting lead ions (Pb^{2+}) in biological matrix. In this way, DNA-functionalized AuNPs were designed for the specific detection of Pb^{2+}.[163] In the presence of Pb^{2+}, the aggregation of the DNA-functionalized AuNPs was triggered for the enhancement of the peroxidase-mimetic activity of AuNPs. Then, the chromogenic reaction of 3,3',5,5'-tetramethylbenzidine (TMB) by the catalyst of AuNPs was used for the sensitive UV-vis and colorimetric detection of Pb^{2+}. Consequently, based on the specific properties of AIE molecules, the AIE-based DNA nanostructures have exhibited advantages in biological applications.

Near-infrared (NIR)-based DNA nanostructures

Although multiple strategies can enhance the responses for sensitive detections, some other factors, such as tissue penetration and phototoxicity, still challenged the *in vivo* biological applications of FDNs. Recently, photochemical-based strategy for constructing DNA nanostructures has been proposed for biomedical applications.However, the requirement of ultraviolet (UV) or blue light in these systems[164-166] significantly limited the biological applications due to associated poor tissue penetration and phototoxicity. Alternatively, the bio-window NIR light has enabled penetration of deeper tissue than UV and visible light, which exhibits satisfied biocompatibility for wider biological applications. Based on the programmable properties of DNA molecules, the NIR-active components can be precisely positioned to enhance the photoactivation efficiency. Furthermore, DNA nanostructures can serve as carriers for NIR-responsive molecules, enabling controlled release of therapeutic agents.[167] Thereby, DNA nanotechnology provides a versatile NIR-photoactivatable probes by incorporating NIR dyes or plasmonic nanoparticles into DNA nanostructures,[168] showing great potential in biomedical applications.

Firstly, the NIR-based DNA nanostructures have exhibited great advantages for *in vivo* imaging. For example, a photo-locked DNA nanomachine (P-DNA) has been designed to spatiotemporally activate nanomachine operation for imaging of metal ions in cancer cells upon single NIR irradiation.[169] Besides, multiple-armed DNA tetrahedral nanostructures (TDNs) were also designed for dual-modality imaging based on NIR fluorescence and single-photon emission computed tomography (SPECT).[170] With this platform, functional groups including tumor-targeting folic acid, NIR emitter Dylight 755, and radioactive isotope ^{99m}Tc were precisely anchored and noninvasively detected in tumor-bearing mice. In another work (Figure 6A), a tetrahedral DNA framework (TDF)-based nanodevice was constructed for *in vivo* NIR

diagnosis of early-stage acute kidney injury (AKI) upon detecting slight histopathologic damages in the kidney.[171] This nanodevice comprised three functional modules: a size-tunable TDF nanostructure as a kidney-targeting vehicle, a binding module for targeting the biomarker kidney injury molecule-1 (Kim-1), and a NIR signaling module. With this nanodevice, the early diagnosis of AKI onset was demonstrated at least 6 h ahead of Kim-1 urinalysis, or 12 h ahead of blood detection.

Notably, fluorescence in the NIR-II region (1000-1700 nm) has a deeper penetration depth and a better signal-to-noise ratio compared to conventional fluorescence in the NIR-I region. Compared to commonly used X-ray computed tomography (CT) and magnetic resonance imaging (MRI), the fluorescence imaging of the IR-II region is convenient and allows for real-time intraoperative monitoring. Recently, IR-II-emitting organic nanofluorophores have been constructed and applied to non-invasive imaging of the brain. For example, a nanocarrier of DNA organic nano-fluorophores was prepared using DNA block copolymer and polystyrene-*b*-DNA. As demonstrated (Figure 6B), this platform can efficiently cross the blood-brain barrier and target brain tumors to emit signals at the NIR-II region, achieving imaging of brain tumors at ultra-high resolution.[172]

In addition, DNA-based nanodevices are also widely used for NIR-controlled drug release and bioimaging. To achieve precise control over the space and duration of drug release, a thiolated pH-responsive DNA conjugated gold nanorod (GNR) was developed. This DNA-based nanodevice acted as a multifunctional nanocarrier for the targeted delivery of drugs, which was triggered by dual-stimuli of low pH and NIR.[173] As demonstrated, the rapid and effective release of chemotherapeutic drug (DOX) was triggered by an acidic pH environment (pH~5) and/or under a NIR laser radiation. Furthermore, exogenous photoacoustic probes also exhibited the NIR absorbance,[174] which initiated photoacoustic imaging for cancer hypoxia diagnosis or sensitive monitoring of tumor treatments. To avoid the effect of tumor hypoxia for chemotherapy, a DNA origami-based theranostic nanoplatform was developed with an intercalated anticancer anthraquinone as both a chemotherapeutic drug and a photoacoustic contrast agent.[175] Furthermore, to avoid uncontrollable target identification, a fluorescent nanosensor was constructed by introducing the nucleic acid confinement effect into a NIR photo-controlled sensing platform.[176] As shown in Figure 6C, the NIR sensing was initiated by an external light irradiation at 808 nm. Simultaneously, the local reaction concentrations were vastly enhanced by a special confinement effect from a six-branched DNA nanowheel, which highly guaranteed sensitive detections. Moreover, to address the limitations associated with current oligonucleotide delivery vehicles, the fluoropolymer-coated DNA nanoclews (FNCs) were constructed for the systemic delivery of oligonucleotides.[177] As shown in Figure 6D, fluorinated zwitterionic monomers were in situ polymerized and attached to DNA nanoclews through hydrogen bonding. As designed, the fluoropolymer coating prolonged circulation time, which facilitated cellular internalization and endosomal escape of DNA nanoclews for the subsequent efficient biological applications.

More interestingly, upconversion nanoparticles (UCNPs)-based FDNs can enable indirect activation of photodynamic therapy (PDT) using NIR light.[178,179] This also provides an excellent alternative strategy for the diagnosis and treatment of deep tumors. For UCNP-based applications, DNA logic circuits were functionally modified on down/upconversion nanoparticles to construct smart down/upconversion nanomachines (D/UCNMs) for NIR light-triggered PDT.[180] In this way, a NIR-activated nanodevice functionalized with UCNPs was reported, acting as spatially and temporally controlled probes for miRNA imaging *in vivo* (Figure 7A).[181] Thereafter, with various aptamers, NIR-based DNA nanodevices can be constructed for sensing of various targets as well as the regulation of biological functions in living systems. For example, a DNA nanodevice was designed to detect ATP in living cells upon up-conversion luminescence. In this system, the fluorescence sensing activity for ATP can be temporarily controlled by NIR irradiation both *in vitro* and *in vivo*.[182] Therefore, the UCNP-based FDN strategy is quite suitable for tumor-specific targeted detection.

The UCNPs-based DNA nanostructures have also exhibited great potential in cancer treatments. For instance, a UCNP-centered Au_{20} -Au_{30} nanoparticle tetrahedron (UAuTe) was prepared by DNA hybridization, which can selectively accelerate the clearance of senescent cells.[183] As shown in Figure 7B, once the senescent cells were recognized by the beta-2-microglobulin antibody (anti-B2MG) on the AuNPs, UAuTe was disassembled by breaking the boronic ester linkage upon NIR-light irradiation. Subsequently, the apoptosis in senescent cells was induced by Granzyme B exposed to the UCNPs, which were tracked by fluorescence changes. In the same year, an upconversion DNA nanoplatform was constructed for DNAzyme-mediated gene silencing of survivin, which achieved enhanced cancer therapy by the combination of NIR-triggered PDT with gene therapy.[184] As shown in Figure 7C, the multivalent ssDNA endowed the upconversion nanoplatform with efficient recognition and high loading of photosensitizers and DNAzymes. When the nanoplatform was targeted and internalized into cancer cells, PDT was triggered by NIR light to generate reactive oxygen species for killing cancer cells. Recently, to achieve controlled and precise release of medicine, a NIR-guided DNA nanodevice was designed by engineering guanine cytosine-rich DNA duplexes with a UV-labile moiety onto UCNPs.[185] As shown in Figure 7D, DOX was incorporated into photolabile DNA duplexes (PD), whose fluorescence was quenched via interacting with the guanine-cytosine motif. Acting as a transducer, UV light was emitted upon NIR-light irradiation of UCNPs. As resulted, photolysis of ortho-nitrobenzyl moiety in PD was induced for controlled release of DOX along with the recovery of fluorescence. Therefore, efficient imaging of tumor cells and the inhabitation of their proliferation were simultaneously achieved in the NIR light-responsive system. Besides, an AS1411-functionalized triangle DNA origami (TOADI) was prepared to deliver both DOX and a photosensitizer (indocyanine green, ICG).[186] As shown in Figure 7E, triggered by NIR laser irradiation, TOADI controllably released the DOX into the nucleus through the photothermal effect of ICG. Simultaneously, upon NIR light irradiation, the UCNPs acted as energy transducers to emit UV light for breaking the photolabile moiety in DNA duplexes. This resulted in the controlled release of drugs and recovery of their fluorescence for monitoring. In conclusion, the NIR-induced DNA nanostructures have provided a practical strategy for clinical diagnosis and therapy.

Small fluorescent dyes-based DNA nanostructures

Inspired by the aforementioned NIR-based DNA nanostructures with NIR molecules as the fluorophores, the investigation of small fluorescent dyes is another aspect to promote the development of FDNs. With the development of DNA techniques, fluorescent dyes can be modified on DNA strands to track the cycling, distribution, and lifetime of biomolecules in living systems.[56] Given the spatiotemporally controlled predictable, programmable and addressable properties of DNA nanostructures, fluorescent dyes along with other functional molecules can be integrated in one DNA nanostructure for simultaneous imaging and delivery. For example, the aptamer-conjugated FRET nanoflowers (NFs) were designed upon modification with fluorescent molecules of fluorescein (FAM), cyanine 3 (Cy3), and 6-carboxyl-X-rhodamine (ROX). As designed, an efficient FRET system from intercalated dyes to covalently attached acceptor dyes exhibited 10-fold higher absorption efficiency than the individual ones. Consequently, the NFs exhibited high fluorescence intensity and excellent photostability for multiplexed cellular imaging and traceable targeted drug delivery.[187] Thereafter (Figure 8A), dye-intercalated DNA complexes were integrated in organic nanofibers to support optical birefringence with fully reversible switching, controlled by continuous-wave laser irradiation.[188] As convenient and efficient approaches to template molecular organization, the nanofibers evoked new routes for constructing optical logic gates, reconfigurable photonic networks and fabricating sensors.

As reported, helical arrays of dyes can also be assembled using nucleic acid and polypeptide templates.[189,190] The supramolecular arrays can also be prepared based on the self-assembly of the molecular components.[191] The easy synthesis of noncovalent assemblies was appealing, although it generally lacked precision covalent structures. For the easy preparation, Armitage's group has synthesized supramolecular complexes consisting of a branched DNA template and fluorogenic intercalating dyes.[192] As shown in Figure 8B, the dyes can be intercalated into each base pair, obtaining high-density fluorophores upon assembly of DNAs. At the same time, the DNA template kept them far enough away from each other, efficiently preventing the self-quenching. These properties of small fluorescent dyes further enabled the advanced application of DNA nanostructures.

In addition, various DNA nanostructures have been synthesized and widely

used in the delivery of small molecular drugs. Among the anticancer drugs, doxorubicin hydrochloride (DOX) and camptothecin (CPT) are most widely used in chemotherapy with fewer side effects. For delivery of small molecular drugs, the binding of DOX molecules to TDN-17 under different conditions was investigated by combining molecular docking and all-atom molecular dynamics simulations (Figure 8C).[193] Interestingly, the binding of DOX molecules with a DNA carrier not only facilitated fluorescent imaging, but also inhibited the macromolecular biosynthesis.[194] However, the luminous wavelength of DOX is not long enough, and the following problems still exist in biological imaging: 1) The penetration ability of biological tissue is not strong enough; 2) The interference of animal autofluorescence at this wavelength is very serious. 3) The self-quenching of DOX is very serious, and the fluorescence efficiency is relatively low. Consequently, further developments are still encouraged for the delivery of other fluorescent drugs by DNA nanostructures.

For delivery of other fluorescent drugs, a precise fluorescent drug-containing DNA framework was constructed for cancer treatment based on DNA's excellent programmability and double helix conformation.[195] As shown in Figure 8D, carbonethyl bromide-modified CPT directly reacted with phosphorothioate (PS) that modified DNAs, resulting in the formation of chemotherapeutics-grafted DNAs with a responsive disulfide linkage. This drug-carrying DNA tetrahedral framework has exhibited fluorescence properties and stimulus-response properties, which enhanced anti-tumor efficacy *in vivo* and *in vitro*.

Although the introducing of small fluorescent dyes into DNA nanostructures facilitated real-time visualization of efficient diagnosis, the biological applications could still be challenged by the toxicity and stability of small fluorescent dyes.[196] The toxicity of small fluorescent dyes could be induced by various factors, including their chemical structure, concentration, and interaction with cellular components. Firstly, some fluorescent dyes could disrupt cellular processes, interfere with DNA replication or transcription, or induce oxidative stress. Secondly, small fluorescent dyes could also challenge the stability of DNA nanostructures. Normally, photobleaching could be induced for losing fluorescence intensity of fluorescent dyes upon repeated excitations, limiting their performance of long-term imaging by DNA nanostructures. Additionally, small dyes could be altered upon changes of pH, temperature or the presence of reactive molecules, which could lead to degradations or changes of fluorescence properties. Consequently, the precise design of small fluorescent dye-based DNA nanostructures is required to ensure the low toxicity and stability for reliable biological applications.

To decrease toxicity and increase stability of the small dye-based DNA nanostructures, the precise and powerful design of DNA nanostructures have been arisen for self-assembly of DNA nanostructures and fluorescent dyes. For example, some fluorescent molecules/dyes, such as DOX and CPT, can dwell themselves in DNA by intercalating the GC-rich regions of the double helices. Additionally, special modifications on small fluorescent dyes have also been employed to reduce their toxicity or increase their stability. Upon incorporating specific functional groups, their interaction with cellular components can be minimized and their resistance to degradation can also be enhanced. Meanwhile, encapsulating the dyes within protective coatings or nanoparticles can also avoid the interface of environmental factors and minimize their toxicity. In this way, DOX-loaded DNA origami exhibited remarkable antitumor efficacy without observable toxicity for nude mice with orthotopic breast tumors.[197] Therefore, upon several modification strategies, small dye-based DNA nanostructures could be innovative platforms for the efficient and low-toxic cancer therapeutics *in vivo*.

Surface plasmon resonance (SPR)-based DNA nanostructures

Some other optical techniques could also be introduced into DNA nanostructure engineering. For example, SPR-sensing technology is a versatile technique for constructing optical sensing platforms.[198,199] In another words, the DNA nanostructures can devote a significant enhancement on the sensitivity and selectivity of SPR-based detections. For example, functionalized with thiol-modified DNA strands, DNA nanostructures were immobilized onto gold surfaces. Consequently, the specific interaction between DNA and gold surfaces formed a stable and well-defined DNA monolayer, facilitating the accurate detection of biomolecular interactions through SPR.[200] Besides, the programmability of DNA allowed for the design of DNA nanostructures with tailored surface densities and orientations, further optimizing the performance of SPR-based assays.[201] In fact, DNA nanotechnology enabled the incorporation of various functional elements, such as aptamers or enzymes, into the DNA nanostructure, expanding the scope of SPR-based detection methods.

Furthermore, the gene editing technologies can also be introduced into SPR-based detection of nucleic acids. For example, a "gene scissors" of clustered regularly interspaced short palindromic repeat (CRISPR) platform[204,205] has been proposed to combine with SPR. Recently, a CRISPR-empowered SPR diagnostic strategy was established to fulfill accurate detection of ~ 5 fM unamplified DNA samples.[206] However, dCas9 protein and sgRNA were normally immobilized onto the chip for the detection, making the detection procedures complicated and time-consuming. In addition, the CRISPR's abilities of distinguishing single mutated site in gene sequences was encouraged to be invoked. Accordingly, taking advantage of the mutation site-specific recognizing ability of the CRISPR/Cas12a system, a CRISPR/Cas12a-SPR biosensor was designed to correctly determine the SARS-Cov-2 concentration in both fake viruses and clinical samples (Figure 9A). Upon the lateral (trans-) cleavage properties of the CRISPR/Cas12a, only gold nanoparticle-linked ssDNAs were immobilized on the chip, dramatically simplifying the chip preparations.[202] In addition, a portable biosensor, called the CRISPR-SPR-FT biosensor, was further developed for the detection of MPXV DNA.[203] As shown in Figure 9B, based on the trans-cleavage of target DNA by CRISPR/Cas12a system, the SPR-based fiber probes of ssDNA reporter were activated. Upon the presence of MPXV DNA, Cas12a cleaved the ssDNA reporter molecules for releasing AuNPs. Therefore, the reductive CRISPR reaction was employed on the sensor surface to result the decreased SPR signals. Without the amplification, the dsDNA from MPXV was detected by CRISPR-SPR-FT biosensor within 1.5 h, at a detection limit below 5 aM plasmids. Therefore, integrating sequence-specific recognition of CRISPR/Cas systems with highly sensitive SPR sensors, precise, stable, sensitive and reliable gene analysis of clinical samples would be initiated.

SUMMARY AND OUTLOOK

With the advantages of easy agent-loading, excellent biocompatibility and biodegradability, and low physiological effects, FDNs have exhibited great potential in biomedical applications. However, there are still several challenges that need to be addressed to fully exploit the potential of FDNs in biomedical research and clinical practice. Further efforts could be required for enlarging biological applications of FDNs, such as the following issues:

(1) The design, synthesis and purification of complex FDNs with precise control over shape and functionality are further pursued. Currently, the design of FDNs heavily relies on computational modeling and simulation techniques, which could suffer the low yields and poor reproducibility. Besides, the complexity of structures and some interactions with the environment species could also limit the further applications. Consequently, improving the design and synthesis methods of FDNs will enable the creation of more intricate and functional nanostructures for wider biomedical applications. Series of efforts could address these problems, including exploring new synthesis methods or modification techniques, optimizing reaction conditions, and introducing automated synthesis platforms. Additionally, the development of high-throughput purification techniques could also improve the scalability and reproducibility of the process, such as size-exclusion chromatography or DNA origami purification methods. Besides, some new experimental techniques could further overcome the limitations of computational modeling. For example, upon folding long single-stranded DNA molecules into desired shapes using shorter complementary strands as staples, the DNA origami enables the precise creation of complex DNA nanostructures. Furthermore, other biomolecules, such as proteins or RNA, could also be adopted to conjunct with DNA nanostructures to enhance their functionality. Additionally, the external stimuli, such as light or temperature, could also enable the dynamic control over their shape and functionality. Besides, the seeking of fluorophores with enhanced brightness, photostability, and compatibility would also initiate the outstanding FDNs. Anyway, with the development of nanotechnology, synthesis and nuclear acid engineering, more FDNs with

precise structures and excellent performance would be constructed.

(2) The stability and biocompatibility of FDNs in biological environments should be further improved to ensure their applicability in biological systems. As we know, DNA is susceptible to be degraded by nucleases and other enzymes in biological fluids, which could limit the stability and effectiveness of DNA nanostructures *in vivo*. Additionally, the interaction between DNA nanostructures and biological molecules could also affect their stability and functionality in biological systems. Therefore, further developments of strategies to enhance the stability and biocompatibility of DNA nanostructures will be crucial to ensure their successful translation for efficient biomedical applications. Modifications of the DNA backbones or nucleotide bases, such as phosphorothioate linkages or methyl groups, could enhance the resistance of DNA nanostructures to enzymatic degradations. Another solution is to encapsulate DNA nanostructures within protective coatings or nanoparticles, such as liposomes or polymeric nanoparticles. These coatings can shield the DNA nanostructures from enzymatic degradation, providing additional barrier against interactions with biological molecules. Moreover, some biocompatible materials, such as DNA aptamers or proteins, could also stabilize DNA nanostructures and avoid enzymatic degradations. For obtaining the more biocompatible FDNs, more simple and biocompatible strategies are still encouraged to be further developed for the better biological applications.

(3) The scalability and cost-effectiveness of FDNs are required to be addressed. Despite the low-cost of DNA strands, the cost could still be greatly increased by the introducing of probe-labeled strands (e.g., MB and TaqMan probe) and tool-enzymes. In addition, current methods for the synthesis of DNA nanostructures are normally time-consuming with specialized equipment and expertise. Additionally, the cost associated with the synthesis and purification of DNA nanostructures could be prohibitive, limiting their widespread biomedical and clinical applications. Hence, developing cost-effective and scalable synthesis methods will enable the widespread biological applications of DNA nanostructures. The label-free and enzyme-free methods could be helpful for decreasing the cost of DNA devices. In addition, the self-assemble strategies could also facilitate the construction of complex DNA architectures, which would reduce cost by minimizing the intricate fabrication processes. Along with the developments of the interdisciplinary technologies, the scalability and cost-effective FDNs would be kept on improving for the wider applications.

Despite these challenges, the future biomedical applications of FDNs still hold great promise. As reviewed, FDNs have been endowed with a wide range of functions with the benefits of controllable and precise constructions, opening up new opportunities in multi-modal biological applications. Combined with a variety of biotechnology methods, FDNs keep on improving to obtain more and more sensitive and precise detections with high resolution and signal-to-noise ratios. In addition, efficient disease treatments can be simultaneously achieved along with the sensitive diagnosis. Moreover, the integration of DNA nanostructures with other emerging technologies will further expand their capabilities in biological applications. Consequently, FDNs have become an invaluable platform for biological applications, providing advanced opportunities for sensing, imaging, catalysis, diagnosis and treatments.

REFERENCES

1. Gao, Q., Zeng, Q., Wang, Z., et al. (2022). Circulating cell-free DNA for cancer early detection. The Innovation **3**: 100259. DOI: 10.1016/j.xinn.2022.100259.
2. Seeman, N.C., and Sleiman, H.F. (2017). DNA nanotechnology. Nature Reviews Materials **3**: 17068. DOI: 10.1038/natrevmats.2017.68.
3. Awaja, F., Wakelin, E.A., Sage, J., et al. (2015). Description of DNA molecular motion for nanotechnology applications. Progress in Materials Science **74**: 308–331. DOI: 10.1016/j.pmatsci.2015.03.001.
4. Zhang, F., Nangreave, J., Liu, Y., et al. (2014). Structural DNA nanotechnology: State of the art and future perspective. Journal of the American Chemical Society **136**: 11198–11211. DOI: 10.1021/ja505101a.
5. Aldaye, F.A., Palmer, A.L., and Sleiman, H.F. (2008). Assembling Materials with DNA as the Guide. Science **321**: 1795–1799. DOI: 10.1126/science.1154533.
6. Julin, S., Nummelin, S., Kostiainen, M.A., et al. (2018). DNA nanostructure-directed assembly of metal nanoparticle superlattices. Journal of Nanoparticle Research **20**: 119. DOI: 10.1007/s11051-018-4225-3.
7. Mirkin, C.A., Letsinger, R.L., Mucic, R.C., et al. (1996). A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature **382**: 607–609. DOI: 10.1038/382607a0.
8. Alivisatos, A.P., Johnsson, K.P., Peng, X., et al. (1996). Organization of 'nanocrystal molecules' using DNA. Nature **382**: 609–611. DOI: 10.1038/382609a0.
9. Fu, J., Liu, M., Liu, Y., et al. (2012). Spatially-Interactive biomolecular networks organized by nucleic acid nanostructures. Accounts of Chemical Research **45**: 1215–1226. DOI: 10.1021/ar200295q.
10. Wang, X., Chandrasekaran, A.R., Shen, Z., et al. (2019). Paranemic crossover DNA: There and back again. Chemical Reviews **119**: 6273–6289. DOI: 10.1021/acs.chemrev.8b00207.
11. Loescher, S., Groeer, S., and Walther, A. (2018). 3D DNA origami nanoparticles: From basic design principles to emerging applications in soft matter and (bio-) nanosciences. Angewandte Chemie International Edition **57**: 10436–10448. DOI: 10.1002/anie.201801700.
12. Tørring, T., Voigt, N.V., Nangreave, J., et al. (2011). DNA origami: a quantum leap for self-assembly of complex structures. Chemical Society Reviews **40**: 5636–5646. DOI: 10.1039/C1CS15057J.
13. Hong, F., Zhang, F., Liu, Y., et al. (2017). DNA origami: Scaffolds for creating higher order structures. Chemical Reviews **117**: 12584–12640. DOI: 10.1021/acs.chemrev.6b00825.
14. Rothemund, P.W.K. (2006). Folding DNA to create nanoscale shapes and patterns. Nature **440**: 297–302. DOI: 10.1038/nature04586.
15. Kopperger, E., List, J., Madhira, S., et al. (2018). A self-assembled nanoscale robotic arm controlled by electric fields. Science **359**: 296–301. DOI: 10.1126/science.aao4284.
16. Simmel, F.C., Yurke, B., and Singh, H.R. (2019). Principles and applications of nucleic acid strand displacement reactions. Chemical Reviews **119**: 6326–6369. DOI: 10.1021/acs.chemrev.8b00580.
17. Wang, S.S., and Ellington, A.D. (2019). Pattern generation with nucleic acid chemical reaction networks. Chemical Reviews **119**: 6370–6383. DOI: 10.1021/acs.chemrev.8b00625.
18. Petersen, P., Tikhomirov, G., and Qian, L. (2018). Information-based autonomous reconfiguration in systems of interacting DNA nanostructures. Nature Communications **9**: 5362. DOI: 10.1038/s41467-018-07805-7.
19. Chen, J., Chen, Z., Meng, C., et al. (2023). CRISPR-powered optothermal nanotweezers: Diverse bio-nanoparticle manipulation and single nucleotide identification. Light: Science & Applications **12**: 273. DOI: 10.1038/s41377-023-01326-9.
20. Chen, Z., Wu, C., Yuan, Y., et al. (2023). CRISPR-Cas13a-powered electrochemical biosensor for the detection of the L452R mutation in clinical samples of SARS-CoV-2 variants. Journal of Nanobiotechnology **21**: 141. DOI: 10.1186/s12951-023-01903-5.
21. Shen, J., Chen, Z., Xie, R., et al. (2023). CRISPR/Cas12a-assisted isothermal amplification for rapid and specific diagnosis of respiratory virus on an microfluidic platform. Biosensors and Bioelectronics **237**: 115523. DOI: 10.1016/j.bios.2023.115523.
22. Yang, D., Hartman, M.R., Derrien, T.L., et al. (2014). DNA materials: bridging nanotechnology and biotechnology. Accounts of Chemical Research **47**: 1902–1911. DOI: 10.1021/ar5001082.
23. Li, J., Green, A.A., Yan, H., et al. (2017). Engineering nucleic acid structures for programmable molecular circuitry and intracellular biocomputation. Nature Chemistry **9**: 1056–1067. DOI: 10.1038/nchem.2852.
24. Kohman, R.E., Kunjapur, A.M., Hysolli, E., et al. (2018). From designing the molecules of life to designing life: Future applications derived from advances in DNA technologies. Angewandte Chemie International Edition **57**: 4313–4328. DOI: 10.1002/anie.201707976.
25. Hu, Q., Li, H., Wang, L., et al. (2019). DNA nanotechnology-enabled drug delivery systems. Chemical Reviews **119**: 6459–6506. DOI: 10.1021/acs.chemrev.7b00663.
26. Walter, J.A., Mikhail, Y.B., Hyeran, L., et al. (2010). Biological applications of fluorescence lifetime imaging beyond microscopy. Reporters, Markers, Dyes, Nanoparticles, and Molecular Probes for Biomedical Applications II. SPIE **7576**: 214–222. DOI: 10.1117/12.849453.
27. Yao, J., Yang, M., and Duan, Y. (2014). Chemistry, biology, and medicine of fluorescent nanomaterials and related systems: New insights into biosensing, bioimaging, genomics, diagnostics, and therapy. Chemical Reviews **114**: 6130–6178. DOI: 10.1021/cr200359p.
28. Xing, L., Todd, N.W., Yu, L., et al. (2010). Early detection of squamous cell lung cancer in sputum by a panel of microRNA markers. Modern Pathology **23**: 1157–1164. DOI: 10.1038/modpathol.2010.111.
29. Choi, Y.-E., Kwak, J.-W., and Park, J.W. (2010). Nanotechnology for early cancer detection. Sensors **10**: 428–455. DOI: 10.3390/s100100428.
30. Lu, H., Wang, Y., and Yu, R. (2023). Immune cell membrane-coated nanoparticles for targeted myocardial ischemia/reperfusion injury therapy. The Innovation Medicine **1**: 100015. DOI: 10.59717/j.xinn-med.2023.100015.
31. Cao, C., and Cao, X. (2023). Nanowire-based smart windows achieving dynamic solar radiation regulation. The Innovation Materials **1**: 100024. DOI: 10.59717/j.xinn-mater.2023.100024.
32. Huang, Z., Zeng, S., Wen, S., et al. (2023). Fluorescence-guided visualization of pancreatic neuroendocrine tumor borders. The Innovation Medicine **1**: 100025. DOI:

10.59717/j.xinn-med.2023.100025.
33. Chinen, A.B., Guan, C.M., Ferrer, J.R., et al. (2015). Nanoparticle probes for the detection of cancer biomarkers, cells, and tissues by fluorescence. Chemical Reviews **115**: 10530–10574. DOI: 10.1021/acs.chemrev.5b00321.
34. Berezin, M.Y., and Achilefu, S. (2010). Fluorescence lifetime measurements and biological imaging. Chemical Reviews **110**: 2641–2684. DOI: 10.1021/cr900343z.
35. Hötzer, B., Medintz, I.L., and Hildebrandt, N. (2012). Fluorescence in nanobiotechnology: sophisticated fluorophores for novel applications. Small **8**: 2297–2326. DOI: 10.1002/smll.201200109.
36. Seeman, N.C. (1982). Nucleic acid junctions and lattices. Journal of Theoretical Biology **99**: 237–247. DOI: 10.1016/0022-5193(82)90002-9.
37. Ma, R.-I., Kallenbach, N.R., Sheardy, R.D., et al. (1986). Three-arm nucleic acid junctions are flexible. Nucleic Acids Research **14**: 9745–9753. DOI: 10.1093/nar/14.24.9745.
38. Baig, M.M.F.A., Gao, X., Khan, M.A., et al. (2022). Nanoscale packing of DNA tiles into DNA macromolecular lattices. International Journal of Biological Macromolecules **220**: 520–527. DOI: 10.1016/j.ijbiomac.2022.08.107.
39. Han, D., Qi, X., Myhrvold, C., et al. (2017). Single-stranded DNA and RNA origami. Science **358**: eaao2648. DOI: 10.1126/science.aao2648.
40. Liu, D., Wang, M., Deng, Z., et al. (2004). Tensegrity: Construction of rigid DNA triangles with flexible four-arm DNA junctions. Journal of the American Chemical Society **126**: 2324–2325. DOI: 10.1021/ja031754r.
41. Liu, D., Park, S.H., Reif, J.H., et al. (2004). DNA nanotubes self-assembled from triple-crossover tiles as templates for conductive nanowires. Proceedings of the National Academy of Sciences **101**: 717–722. DOI: 10.1073/pnas.0305860101.
42. Goodman, R.P., Berry, R.M., and Turberfield, A.J. (2004). The single-step synthesis of a DNA tetrahedron. Chemical Communications(12):1372-1373. DOI: 10.1039/B402293A.
43. Shih, W.M., Quispe, J.D., and Joyce, G.F. (2004). A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron. Nature **427**(6975):618-621. DOI: 10.1038/nature02307.
44. Wang, H., Luo, D., Wang, H., et al. (2021). Construction of smart stimuli-responsive DNA nanostructures for biomedical applications. Chemistry – A European Journal **27**: 3929–3943. DOI: 10.1002/chem.202003145.
45. Tang, J., Yao, C., Gu, Z., et al. (2020). Super-soft and super-elastic DNA robot with magnetically driven navigational locomotion for cell delivery in confined space. Angewandte Chemie International Edition **59**: 2490–2495. DOI: 10.1002/anie.201913549.
46. Gong, X., Li, R., Wang, J., et al. (2020). A smart theranostic nanocapsule for spatiotemporally programmable photo-gene therapy. Angewandte Chemie International Edition **59**: 21648–21655. DOI: 10.1002/anie.202008413.
47. Pogu, S.V., Dehariya, D., Yadav, D.N., et al. (2023). A review on fabrication, actuation, and application of magnetic force driven, light driven and DNA nano/microrobots in modern theranostics. Molecular Systems Design & Engineering **8**: 416–430. DOI: 10.1039/D2ME00247G.
48. Wei, B., Dai, M., and Yin, P. (2012). Complex shapes self-assembled from single-stranded DNA tiles. Nature **485**: 623–626. DOI: 10.1038/nature11075.
49. Zhang, Y., and Seeman, N.C. (1994). Construction of a DNA-truncated octahedron. Journal of the American Chemical Society **116**: 1661–1669. DOI: 10.1021/ja00084a006.
50. Zhou, Y., Dong, J., Zhou, C., et al. (2022). Finite assembly of three-dimensional DNA hierarchical nanoarchitectures through orthogonal and directional bonding. Angewandte Chemie International Edition **61**: e202116416. DOI: 10.1002/anie.202116416.
51. Han, D., Pal, S., Nangreave, J., et al. (2011). DNA origami with complex curvatures in three-dimensional space. Science **332**: 342–346. DOI: 10.1126/science.1202998.
52. Yurke, B., Turberfield, A.J., Mills, A.P., et al. (2000). A DNA-fuelled molecular machine made of DNA. Nature **406**: 605–608. DOI: 10.1038/35020524.
53. Yan, H., Zhang, X., Shen, Z., et al. (2002). A robust DNA mechanical device controlled by hybridization topology. Nature **415**: 62–65. DOI: 10.1038/415062a.
54. Omabegho, T., Sha, R., and Seeman, N.C. (2009). A bipedal DNA brownian motor with coordinated legs. Science **324**: 67–71. DOI: 10.1126/science.1170336.
55. Yan, J., Hu, C., Wang, P., et al. (2015). Growth and origami folding of DNA on nanoparticles for high-efficiency molecular transport in cellular imaging and drug delivery. Angewandte Chemie International Edition **54**: 2431–2435. DOI: 10.1002/anie.201408247.
56. Shen, X., Jiang, Q., Wang, J., et al. (2012). Visualization of the intracellular location and stability of DNA origami with a label-free fluorescent probe. Chemical Communications **48**: 11301–11303. DOI: 10.1039/C2CC36185J.
57. Edwardson, T.G.W., Lau, K.L., Bousmail, D., et al. (2016). Transfer of molecular recognition information from DNA nanostructures to gold nanoparticles. Nature Chemistry **8**: 162–170. DOI: 10.1038/nchem.2420.
58. Li, J., Wei, J., Gao, Y., et al. (2023). Peptide-assembled siRNA nanomicelles confine MnOx-loaded silicages for synergistic chemical and gene-regulated cancer therapy. Chinese Chemical Letters **34**: 107662. DOI: 10.1016/j.cclet.2022.07.005.
59. Rosenzweig, B.A., Ross, N.T., Tagore, D.M., et al. (2009). Multivalent protein binding and precipitation by self-assembling molecules on a DNA pentaplex scaffold. Journal of the American Chemical Society **131**: 5020–5021. DOI: 10.1021/ja809219p.
60. Lan, X., Su, Z., Zhou, Y., et al. (2017). Programmable supra-assembly of a DNA surface adapter for tunable chiral directional self-assembly of gold nanorods. Angewandte Chemie International Edition **56**: 14632–14636. DOI: 10.1002/anie.201709775.
61. Shen, X., Wang, Y., Zhang, Y., et al. (2018). Unique SiO_2 nanourchins enable amplification in living cells for in situ imaging of mRNAs. Advanced Functional Materials **28**: 1803286. DOI: 10.1002/adfm.201803286.
62. Wang, Y., Lu, X., Wu, X., et al. (2022). Chemically modified DNA nanostructures for drug delivery. The Innovation **3**: 100217. DOI: 10.1016/j.xinn.2022.100217.
63. Förster, T. (1948). Zwischenmolekulare energiewanderung und fluoreszenz. Annalen der Physik **437**(1-2): 55–75. DOI: 10.1002/andp.19484370105.
64. Piston, D.W., and Kremers, G.-J. (2007). Fluorescent protein FRET: the good, the bad and the ugly. Trends in Biochemical Sciences **32**: 407–414. DOI: 10.1016/j.tibs.2007.08.003.
65. Shen, X., Xu, W., Ouyang, J., et al. (2022). Fluorescence resonance energy transfer-based nanomaterials for the sensing in biological systems. Chinese Chemical Letters **33**: 4505–4516. DOI: 10.1016/j.cclet.2021.12.061.
66. Yang, L., Liu, Y., Ma, C., et al. (2015). Naphthalene-fused BODIPY with large Stokes shift as saturated-red fluorescent dye for living cell imaging. Dyes and Pigments **122**: 1–5. DOI: 10.1016/j.dyepig.2015.06.006.
67. Guo, B., Nie, H., Yang, W., et al. (2016). A highly sensitive and rapidly responding fluorescent probe with a large Stokes shift for imaging intracellular hypochlorite. Sensors and Actuators B: Chemical **236**: 459–465. DOI: 10.1016/j.snb.2016.06.004.
68. Gao, Z., Hao, Y., Zheng, M., et al. (2017). A fluorescent dye with large Stokes shift and high stability: synthesis and application to live cell imaging. RSC Advances **7**: 7604–7609. DOI: 10.1039/C6RA27547H.
69. Zhang, D., Wen, Y., Xiao, Y., et al. (2008). Bulky 4-tritylphenylethynyl substituted boradiazaindacene: pure red emission, relatively large Stokes shift and inhibition of self-quenching. Chemical Communications (39):4777-4779. DOI: 10.1039/B808681H.
70. Sednev, M.V., Belov, V.N., and Hell, S.W. (2015). Fluorescent dyes with large Stokes shifts for super-resolution optical microscopy of biological objects: A review. Methods and Applications in Fluorescence **3**: 042004. DOI: 10.1088/2050-6120/3/4/042004.
71. Lin, W., Yuan, L., Cao, Z., et al. (2010). Through-bond energy transfer cassettes with minimal spectral overlap between the donor emission and acceptor absorption: Coumarin–rhodamine dyads with large pseudo-stokes shifts and emission shifts. Angewandte Chemie International Edition **49**: 375–379. DOI: 10.1002/anie.200904515.
72. Zhang, H., Li, H., Cao, Z., et al. (2020). Investigation of the in vivo integrity of polymeric micelles via large Stokes shift fluorophore-based FRET. Journal of Controlled Release **324**: 47–54. DOI: 10.1016/j.jconrel.2020.04.046.
73. Jungmann, R., Avendaño, M.S., Woehrstein, J.B., et al. (2014). Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and exchange-PAINT. Nature Methods **11**: 313–318. DOI: 10.1038/nmeth.2835.
74. Ma, Y., Song, M., Li, L., et al. (2024). Attomolar-level detection of respiratory virus long-chain oligonucleotides based on FRET biosensor with upconversion nanoparticles and Au—Au dimer. Biosensors and Bioelectronics **243**: 115778. DOI: 10.1016/j.bios.2023.115778.
75. Farag, N., Mattossovich, R., Merlo, R., et al. (2021). Folding-upon-repair DNA nanoswitches for monitoring the activity of DNA repair enzymes. Angewandte Chemie International Edition **60**: 7283–7289. DOI: 10.1002/anie.202016223.
76. Li, L., Wang, J., Jiang, H., et al. (2023). DNA tetrahedron-based split aptamer probes for reliable imaging of ATP in living cells. Chinese Chemical Letters **34**: 107506. DOI: 10.1016/j.cclet.2022.05.020.
77. Huizenga, D.E., and Szostak, J.W. (1995). A DNA aptamer that binds adenosine and ATP. Biochemistry **34**: 656–665. DOI: 10.1021/bi00002a033.
78. Nutiu, R., and Li, Y. (2003). Structure-switching signaling aptamers. Journal of the American Chemical Society **125**: 4771–4778. DOI: 10.1021/ja028962o.
79. Di, Z., Zhao, J., Chu, H., et al. (2019). An Acidic-microenvironment-driven DNA nanomachine enables specific ATP imaging in the extracellular milieu of tumor. Advanced Materials **31**: 1901885. DOI: 10.1002/adma.201901885.
80. Carrasquilla, C., Kapteyn, E., Li, Y., et al. (2017). Sol–gel-derived biohybrid materials incorporating long-chain DNA aptamers. Angewandte Chemie International Edition **56**: 10686–10690. DOI: 10.1002/anie.201702859.
81. Zheng, J., Wang, Q., Shi, L., et al. (2021). Logic-gated proximity aptasensing for cell-surface real-time monitoring of apoptosis. Angewandte Chemie International Edition **60**: 20858–20864. DOI: 10.1002/anie.202106651.
82. Xiang, Z., Zhao, J., Qu, J., et al. (2022). A multivariate-gated DNA nanodevice for spatioselective imaging of pro-metastatic targets in extracellular microenvironment. Angewandte Chemie International Edition **61**: e202111836. DOI: 10.1002/anie.202111836.
83. Zhou, X., Zhao, M., Duan, X., et al. (2017). Collapse of DNA tetrahedron nanostructure for "off–on" fluorescence detection of DNA methyltransferase activity. ACS Applied Materials & Interfaces **9**: 40087–40093. DOI: 10.1021/acsami.7b13551.
84. Zhu, G., Zhang, S., Song, E., et al. (2013). Building fluorescent DNA nanodevices on target living cell surfaces. Angewandte Chemie International Edition **52**: 5490–5496.

DOI: 10.1002/anie.201301439.
85. He, Y., and Liu, D.R. (2010). Autonomous multistep organic synthesis in a single isothermal solution mediated by a DNA walker. Nature Nanotechnology **5**: 778–782. DOI: 10.1038/nnano.2010.190.
86. Xu, Z., Dong, Y., Li, J., et al. (2015). A ferrocene-switched electrochemiluminescence "off–on" strategy for the sensitive detection of cardiac troponin I based on target transduction and a DNA walking machine. Chemical Communications **51**: 14369–14372. DOI: 10.1039/C5CC04745E.
87. Wang, L., Deng, R., and Li, J. (2015). Target-fueled DNA walker for highly selective miRNA detection. Chemical Science **6**: 6777–6782. DOI: 10.1039/C5SC02784E.
88. Wickham, S.F.J., Endo, M., Katsuda, Y., et al. (2011). Direct observation of stepwise movement of a synthetic molecular transporter. Nature Nanotechnology **6**: 166–169. DOI: 10.1038/nnano.2010.284.
89. Tomov, T.E., Tsukanov, R., Glick, Y., et al. (2017). DNA bipedal motor achieves a large number of steps due to operation using microfluidics-based interface. ACS Nano **11**: 4002–4008. DOI: 10.1021/acsnano.7b00547.
90. Qu, X., Zhu, D., Yao, G., et al. (2017). An exonuclease III-powered, on-particle stochastic DNA walker. Angewandte Chemie International Edition **56**: 1855–1858. DOI: 10.1002/anie.201611777.
91. Yang, X., Tang, Y., Mason, S.D., et al. (2016). Enzyme-powered three-dimensional DNA nanomachine for DNA walking, payload release, and biosensing. ACS Nano **10**: 2324–2330. DOI: 10.1021/acsnano.5b07102.
92. Xu, Z.-Q., Zhang, P., Chai, Y.-Q., et al. (2018). A biosensor based on a 3D-DNA walking machine network and distance-controlled electrochemiluminescence energy transfer for ultrasensitive detection of tenascin C and lead ions. Chemical Communications **54**: 8741–8744. DOI: 10.1039/C8CC04953J.
93. Li, D., Zhou, W., Yuan, R., et al. (2017). A DNA-fueled and catalytic molecule machine lights up trace under-expressed microRNAs in living cells. Analytical Chemistry **89**: 9934–9940. DOI: 10.1021/acs.analchem.7b02247.
94. Ye, S., Li, X., Wang, M., et al. (2017). Fluorescence and SERS imaging for the simultaneous absolute quantification of multiple miRNAs in living cells. Analytical Chemistry **89**: 5124–5130. DOI: 10.1021/acs.analchem.7b00697.
95. Zhang, H., Lai, M., Zuehlke, A., et al. (2015). Binding-induced DNA nanomachines triggered by proteins and nucleic acids. Angewandte Chemie International Edition **54**: 14326–14330. DOI: 10.1002/anie.201506312.
96. Luan, M., Li, N., Pan, W., et al. (2017). Simultaneous detection of multiple targets involved in the PI3K/AKT pathway for investigating cellular migration and invasion with a multicolor fluorescent nanoprobe. Chemical Communications **53**: 356–359. DOI: 10.1039/C6CC07605J.
97. Chen, J., Zuehlke, A., Deng, B., et al. (2017). A target-triggered DNAzyme motor enabling homogeneous, amplified detection of proteins. Analytical Chemistry **89**: 12888–12895. DOI: 10.1021/acs.analchem.7b03529.
98. Liang, C.-P., Ma, P.-Q., Liu, H., et al. (2017). Rational engineering of a dynamic, entropy-driven DNA nanomachine for intracellular microRNA imaging. Angewandte Chemie International Edition **56**: 9077–9081. DOI: 10.1002/anie.201704147.
99. Ma, P.-Q., Liang, C.-P., Zhang, H.-H., et al. (2018). A highly integrated DNA nanomachine operating in living cells powered by an endogenous stimulus. Chemical Science **9**: 3299–3304. DOI: 10.1039/C8SC00049B.
100. Mason, S.D., Wang, G.A., Yang, P., et al. (2019). Probing and controlling dynamic interactions at biomolecule–nanoparticle interfaces using stochastic DNA walkers. ACS Nano **13**: 8106–8113. DOI: 10.1021/acsnano.9b03053.
101. Kong, L., Kou, B., Zhang, X., et al. (2021). A core–brush 3D DNA nanostructure: the next generation of DNA nanomachine for ultrasensitive sensing and imaging of intracellular microRNA with rapid kinetics. Chemical Science **12**: 15953–15959. DOI: 10.1039/D1SC04571G.
102. Liu, J., and Lu, Y. (2002). FRET study of a trifluorophore-labeled DNAzyme. Journal of the American Chemical Society **124**: 15208–15216. DOI: 10.1021/ja027647z.
103. Deng, R., Yang, H., Dong, Y., et al. (2018). Temperature-robust DNAzyme biosensors confirming ultralow background detection. ACS Sensors **3**: 2660–2666. DOI: 10.1021/acssensors.8b01122.
104. Wu, Z., Fan, H., Satyavolu, N.S.R., et al. (2017). Imaging endogenous metal ions in living cells using a DNAzyme–catalytic hairpin assembly probe. Angewandte Chemie International Edition **56**: 8721–8725. DOI: 10.1002/anie.201703540.
105. Peng, H., Li, X.-F., Zhang, H., et al. (2017). A microRNA-initiated DNAzyme motor operating in living cells. Nature Communications **8**: 14378. DOI: 10.1038/ncomms14378.
106. Wu, M., Zhang, M., Fan, Z., et al. (2021). Ultrasensitive DNA methyltransferase activity sensing and inhibitor evaluation with highly photostable upconversion nanoparticle transducer. Microchimica Acta **188**: 169. DOI: 10.1007/s00604-021-04831-z.
107. Cha, T.-G., Pan, J., Chen, H., et al. (2015). Design principles of DNA enzyme-based walkers: Translocation kinetics and photoregulation. Journal of the American Chemical Society **137**: 9429–9437. DOI: 10.1021/jacs.5b05522.
108. Cha, T.-G., Pan, J., Chen, H., et al. (2014). A synthetic DNA motor that transports nanoparticles along carbon nanotubes. Nature Nanotechnology **9**: 39–43. DOI: 10.1038/nnano.2013.257.
109. Yang, K., Wang, H., Ma, N., et al. (2018). Programmable target-initiated DNAzyme walker walking along a spatially isolated and highly hybridizable substrate track on a nanoparticle surface. ACS Applied Materials & Interfaces **10**: 44546–44553. DOI: 10.1021/acsami.8b16408.
110. Liu, C., Hu, Y., Pan, Q., et al. (2019). A microRNA-triggered self-powered DNAzyme walker operating in living cells. Biosensors and Bioelectronics **136**: 31–37. DOI: 10.1016/j.bios.2019.04.031.
111. Liu, C., Hu, Y., Pan, Q., et al. (2020). A photocontrolled and self-powered bipedal DNA walking machine for intracellular microRNA imaging. Chemical Communications **56**: 3496–3499. DOI: 10.1039/D0CC00017E.
112. Dyla, M., Andersen, J.L., Kjaergaard, M., et al. (2016). Engineering a prototypic P-type ATPase listeria monocytogenes Ca^{2+}-ATPase 1 for single-molecule FRET studies. Bioconjugate Chemistry **27**: 2176–2187. DOI: 10.1021/acs.bioconjchem.6b00387.
113. Fijen, C., Montón Silva, A., Hochkoeppler, A., et al. (2017). A single-molecule FRET sensor for monitoring DNA synthesis in real time. Physical Chemistry Chemical Physics **19**: 4222–4230. DOI: 10.1039/C6CP05919H.
114. Koo, H., Park, I., Lee, Y., et al. (2016). Visualization and quantification of microRNA in a single cell using atomic force microscopy. Journal of the American Chemical Society **138**: 11664–11671. DOI: 10.1021/jacs.6b05048.
115. Wei, J., Gong, X., Wang, Q., et al. (2018). Construction of an autonomously concatenated hybridization chain reaction for signal amplification and intracellular imaging. Chemical Science **9**: 52–61. DOI: 10.1039/C7SC03939E.
116. Gao, R., Hao, C., Xu, L., et al. (2018). Spiny nanorod and upconversion nanoparticle satellite assemblies for ultrasensitive detection of messenger RNA in living cells. Analytical Chemistry **90**: 5414–5421. DOI: 10.1021/acs.analchem.8b00617.
117. Xu, L., Gao, Y., Kuang, H., et al. (2018). MicroRNA-directed intracellular self-assembly of chiral nanorod dimers. Angewandte Chemie International Edition **57**: 10544–10548. DOI: 10.1002/anie.201805640.
118. Ma, W., Sun, M., Fu, P., et al. (2017). A chiral-nanoassemblies-enabled strategy for simultaneously profiling surface glycoprotein and microRNA in living cells. Advanced Materials **29**: 1703410. DOI: 10.1002/adma.201703410.
119. Dai, W., Dong, H., Guo, K., et al. (2018). Near-infrared triggered strand displacement amplification for MicroRNA quantitative detection in single living cells. Chemical Science **9**: 1753–1759. DOI: 10.1039/c7sc04243d.
120. Meng, X., Dai, W., Zhang, K., et al. (2018). Imaging multiple microRNAs in living cells using ATP self-powered strand-displacement cascade amplification. Chemical Science **9**: 1184–1190. DOI: 10.1039/C7SC04725H.
121. Meng, X., Zhang, K., Dai, W., et al. (2018). Multiplex microRNA imaging in living cells using DNA-capped-Au assembled hydrogels. Chemical Science **9**: 7419–7425. DOI: 10.1039/C8SC02858C.
122. Shen, X., Zhang, Y., Sun, J., et al. (2019). Biodegradable nanosyringes for intracellular amplification-based dual-diagnosis and gene therapy in single living cells††Electronic supplementary information (ESI) available: Experimental details, additional data and graphs. Chemical Science **10**(24): 6113-6119. DOI: 10.1039/c9sc01894h.
123. Liu, Y., Chen, Q., Liu, J., et al. (2017). Design of a modular DNA triangular-prism sensor enabling ratiometric and multiplexed biomolecule detection on a single microbead. Analytical Chemistry **89**: 3590–3596. DOI: 10.1021/acs.analchem.6b04918.
124. Yin, P., Choi, H.M.T., Calvert, C.R., et al. (2008). Programming biomolecular self-assembly pathways. Nature **451**: 318–322. DOI: 10.1038/nature06451.
125. Chandran, H., Rangnekar, A., Shetty, G., et al. (2013). An autonomously self-assembling dendritic DNA nanostructure for target DNA detection. Biotechnology Journal **8**: 221–227. DOI: 10.1002/biot.201100499.
126. Xuan, F., and Hsing, I.M. (2014). Triggering hairpin-free chain-branching growth of fluorescent DNA dendrimers for nonlinear hybridization chain reaction. Journal of the American Chemical Society **136**: 9810–9813. DOI: 10.1021/ja502904s.
127. Di, Z., Lu, X., Zhao, J., et al. (2022). Mild acidosis-directed signal amplification in tumor microenvironment via spatioselective recruitment of DNA amplifiers. Angewandte Chemie International Edition **61**: e202205436. DOI: 10.1002/anie.202205436.
128. Peng, Y., Pang, H., Gao, Z., et al. (2023). Kinetics-accelerated one-step detection of microRNA through spatially localized reactions based on DNA tile self-assembly. Biosensors and Bioelectronics **222**: 114932. DOI: 10.1016/j.bios.2022.114932.
129. Peng, Y., Gao, Z., Qiao, B., et al. (2023). Size-controlled DNA tile self-assembly nanostructures through caveolae-mediated endocytosis for signal-amplified imaging of microRNAs in living cells. Advanced Science **10**: 2300614. DOI: 10.1002/advs.202300614.
130. Zhou, Z., Lin, N., Ouyang, Y., et al. (2023). Cascaded, feedback-driven, and spatially localized emergence of constitutional dynamic networks driven by enzyme-free catalytic DNA circuits. Journal of the American Chemical Society **145**: 12617–12629. DOI: 10.1021/jacs.3c02083.
131. Zhou, X.-M., Zhuo, Y., Yuan, R., et al. (2023). Target-mediated self-assembly of DNA networks for sensitive detection and intracellular imaging of APE1 in living cells. Chemical Science **14**: 2318–2324. DOI: 10.1039/D2SC06968G.
132. Li, J., Wang, J., Liu, S., et al. (2020). Amplified FRET nanoflares: An endogenous mRNA-powered nanomachine for intracellular microRNA imaging. Angewandte Chemie International Edition **59**: 20104–20111. DOI: 10.1002/anie.202008245.
133. Chen, Y., Dai, W., Wang, D., et al. (2022). A cancer cell membrane vesicle-packaged DNA nanomachine for intracellular microRNA imaging. Chemical Communications

58: 9488–9491. DOI: 10.1039/D2CC03068C.
134. Lu, H., Yang, F., Liu, B., et al. (2019). Intracellular low-abundance microRNA imaging by a NIR-assisted entropy-driven DNA system. Nanoscale Horizons 4: 472–479. DOI: 10.1039/C8NH00330K.
135. Liu, F., Li, N., Shang, Y., et al. (2022). A DNA-based plasmonic nanodevice for cascade signal amplification. Angewandte Chemie International Edition 61: e202114706. DOI: 10.1002/anie.202114706.
136. Wang, C., Xie, Y., Song, X., et al. (2023). A NIR programmable in vivo miRNA magnifier for NIR-II imaging of early stage cancer. Angewandte Chemie International Edition 62: e202312665. DOI: 10.1002/anie.202312665.
137. Shustova, N.B., Ong, T.-C., Cozzolino, A.F., et al. (2012). Phenyl ring dynamics in a tetraphenylethylene-bridged metal–organic framework: Implications for the mechanism of aggregation-induced emission. Journal of the American Chemical Society 134: 15061–15070. DOI: 10.1021/ja306042w.
138. Ding, D., Li, K., Liu, B., et al. (2013). Bioprobes based on AIE fluorogens. Accounts of Chemical Research 46: 2441–2453. DOI: 10.1021/ar3003464.
139. Mei, J., Huang, Y., and Tian, H. (2018). Progress and trends in AIE-based bioprobes: A brief overview. ACS Applied Materials & Interfaces 10: 12217–12261. DOI: 10.1021/acsami.7b14343.
140. Mei, J., Leung, N.L.C., Kwok, R.T.K., et al. (2015). Aggregation-induced emission: Together we shine, united we soar! Chemical Reviews 115(21):11718-11940. DOI: 10.1021/acs.chemrev.5b00263.
141. Jia, Y., Zuo, X., Lou, X., et al. (2015). Rational designed bipolar, conjugated polymer-DNA composite beacon for the sensitive detection of proteins and ions. Analytical Chemistry 87: 3890–3894. DOI: 10.1021/ac504690y.
142. Zhang, M., Saha, M.L., Wang, M., et al. (2017). Multicomponent platinum(II) cages with tunable emission and amino acid sensing. Journal of the American Chemical Society 139: 5067–5074. DOI: 10.1021/jacs.6b12536.
143. Liu, Y., Deng, C., Tang, L., et al. (2011). Specific detection of d-glucose by a tetraphenylethene-based fluorescent sensor. Journal of the American Chemical Society 133: 660–663. DOI: 10.1021/ja107086y.
144. Li, Y., Kwok, R.T.K., Tang, B.Z., et al. (2013). Specific nucleic acid detection based on fluorescent light-up probe from fluorogens with aggregation-induced emission characteristics. RSC Advances 3: 10135–10138. DOI: 10.1039/C3RA41983E.
145. Ou, X., Hong, F., Zhang, Z., et al. (2017). A highly sensitive and facile graphene oxide-based nucleic acid probe: Label-free detection of telomerase activity in cancer patient's urine using AIEgens. Biosensors and Bioelectronics 89: 417–421. DOI: 10.1016/j.bios.2016.05.035.
146. Hong, Y., Lam, J.W.Y., and Tang, B.Z. (2011). Aggregation-induced emission. Chemical Society Reviews 40: 5361–5388. DOI: 10.1039/C1CS15113D.
147. Hong, Y., Xiong, H., Lam, J.W.Y., et al. (2010). Fluorescent bioprobes: Structural matching in the docking processes of aggregation-induced emission fluorogens on DNA surfaces. Chemistry – A European Journal 16: 1232–1245. DOI: 10.1002/chem.200900778.
148. Li, W., Zhang, Y., Wang, Y., et al. (2021). Nucleic acids induced peptide-based AIE nanoparticles for fast cell imaging. Chinese Chemical Letters 32: 1571–1574. DOI: 10.1016/j.cclet.2020.09.054.
149. Tong, H., Hong, Y., Dong, Y., et al. (2007). Protein detection and quantitation by tetraphenylethene-based fluorescent probes with aggregation-induced emission characteristics. The Journal of Physical Chemistry B 111: 11817–11823. DOI: 10.1021/jp073147m.
150. Wang, M., Zhang, D., Zhang, G., et al. (2008). The convenient fluorescence turn-on detection of heparin with a silole derivative featuring an ammonium group. Chemical Communications 37:4469-4471. DOI: 10.1039/B808877B.
151. Yuan, Y., Zhang, C.-J., and Liu, B. (2015). A photoactivatable AIE polymer for light-controlled gene delivery: Concurrent endo/lysosomal escape and DNA unpacking. Angewandte Chemie International Edition 54: 11419–11423. DOI: 10.1002/anie.201503640.
152. Zhang, C., Hao, L., Calabrese, C.M., et al. (2015). Biodegradable DNA-brush block copolymer spherical nucleic acids enable transfection agent-free intracellular gene regulation. Small 11: 5360–5368. DOI: 10.1002/smll.201501573.
153. Würthner, F. (2020). Aggregation-induced emission (AIE): A historical perspective. Angewandte Chemie International Edition 59: 14192–14196. DOI: 10.1002/anie.202007525.
154. Yu, Y., Liu, J., Zhao, Z., et al. (2012). Facile preparation of non-self-quenching fluorescent DNA strands with the degree of labeling up to the theoretic limit. Chemical Communications 48: 6360–6362. DOI: 10.1039/C2CC32038J.
155. Li, S., Langenegger, S.M., and Häner, R. (2013). Control of aggregation-induced emission by DNA hybridization. Chemical Communications 49: 5835–5837. DOI: 10.1039/C3CC42706D.
156. Ucar, H., and Wagenknecht, H.-A. (2021). DNA-templated control of chirality and efficient energy transport in supramolecular DNA architectures with aggregation-induced emission. Chemical Science 12: 10048–10053. DOI: 10.1039/D1SC02351A.
157. Jiang, M., Gu, X., Lam, J.W.Y., et al. (2017). Two-photon AIE bio-probe with large Stokes shift for specific imaging of lipid droplets. Chemical Science 8: 5440–5446. DOI: 10.1039/C7SC01400G.
158. Gao, Y., He, Z., He, X., et al. (2019). Dual-color emissive AIEgen for specific and label-free double-stranded DNA recognition and single-nucleotide polymorphisms detection. Journal of the American Chemical Society 141: 20097–20106. DOI: 10.1021/jacs.9b09239.
159. Nobe, S., Yamamoto, K., Miyakawa, H., et al. (2023). Sulfonium-cation-containing aggregation-induced emission block copolymers: self-assembly, multicolor emission, and detection for DNA polyplexes. European Polymer Journal 197: 112359. DOI: 10.1016/j.eurpolymj.2023.112359.
160. Chen, Z., Wei, Z., Xiao, F., et al. (2022). The hydrophobicity of AIE dye facilitates DNA condensation for carrier-free gene therapy. Advanced Functional Materials 32: 2207845. DOI: 10.1002/adfm.202207845.
161. Tang, X., Zhu, Y., Guan, W., et al. (2023). Assembling aggregation-induced emission with natural DNA to maximize donor/acceptor ratio for efficient light-harvesting antennae. Aggregate 4: e348. DOI: 10.1002/agt2.348.
162. Li, M., Cai, Y.-N., Peng, C.-F., et al. (2021). DNA dendrimer–templated copper nanoparticles: self-assembly, aggregation-induced emission enhancement and sensing of lead ions. Microchimica Acta 188: 346. DOI: 10.1007/s00604-021-04967-y.
163. Liu, H., Zhang, Y., Xiong, W., et al. (2023). Aggregation-induced enhancement of peroxidase-mimetic activity of DNAzyme-gold nanoparticles for ultrasensitive detection of lead ions. Analytical Methods 15: 4972–4979. DOI: 10.1039/D3AY00399J.
164. Liu, Z., Liu, Y., Chang, Y., et al. (2016). Nanoscale optomechanical actuators for controlling mechanotransduction in living cells. Nature Methods 13: 143–146. DOI: 10.1038/nmeth.3689.
165. Hwang, K., Wu, P., Kim, T., et al. (2014). Photocaged DNAzymes as a general method for sensing metal ions in living cells. Angewandte Chemie International Edition 53: 13798–13802. DOI: 10.1002/anie.201408333.
166. Wang, W., Satyavolu, N.S.R., Wu, Z., et al. (2017). Near-infrared photothermally activated DNAzyme–gold nanoshells for imaging metal ions in living cells. Angewandte Chemie International Edition 56: 6798–6802. DOI: 10.1002/anie.201701325.
167. Gao, W., Fan, X., Bi, Y., et al. (2022). Preparation of NIR-responsive gold nanocages as efficient carrier for controlling release of EGCG in anticancer application. Frontiers in Chemistry 10. DOI: 10.3389/fchem.2022.926002.
168. Ye, M., Kong, Y., Zhang, C., et al. (2021). Near-infrared light controllable DNA walker driven by endogenous adenosine triphosphate for in situ spatiotemporal imaging of intracellular microRNA. ACS Nano 15: 14253–14262. DOI: 10.1021/acsnano.1c02229.
169. Li, Y., Xie, Y., Zhang, Y., et al. (2022). DNA nanomachine activation and Zn^{2+} imaging in living cells with single NIR irradiation. Analytica Chimica Acta 1221: 340149. DOI: 10.1016/j.aca.2022.340149.
170. Jiang, D., Sun, Y., Li, J., et al. (2016). Multiple-armed tetrahedral DNA nanostructures for tumor-targeting, dual-modality in vivo imaging. ACS Applied Materials & Interfaces 8: 4378–4384. DOI: 10.1021/acsami.5b10792.
171. Ding, F., Zhang, S., Liu, S., et al. (2022). Molecular visualization of early-stage acute kidney injury with a DNA framework nanodevice. Advanced Science 9: 2105947. DOI: 10.1002/advs.202105947.
172. Xiao, F., Lin, L., Chao, Z., et al. (2020). Organic spherical nucleic acids for the transport of a NIR-II-emitting dye across the blood–brain barrier. Angewandte Chemie International Edition 59: 9702–9710. DOI: 10.1002/anie.202002312.
173. Zhang, W., Wang, F., Wang, Y., et al. (2016). PH and near-infrared light dual-stimuli responsive drug delivery using DNA-conjugated gold nanorods for effective treatment of multidrug resistant cancer cells. Journal of Controlled Release 232: 9–19. DOI: 10.1016/j.jconrel.2016.04.001.
174. Zeng, L., Ma, G., Lin, J., et al. (2018). Photoacoustic probes for molecular detection: Recent advances and perspectives. Small 14: 1800782. DOI: 10.1002/smll.201800782.
175. Zeng, Y., Chang, P., Ma, J., et al. (2022). DNA origami–anthraquinone hybrid nanostructures for in vivo quantitative monitoring of the progression of tumor hypoxia affected by chemotherapy. ACS Applied Materials & Interfaces 14: 6387–6403. DOI: 10.1021/acsami.1c22620.
176. Liu, J.-X., Xin, M.-K., Sun, X., et al. (2023). NIR photocontrolled fluorescent nanosensor under a six-branched DNA nanowheel-Induced nucleic acid confinement effect for high-performance bioimaging. ACS Applied Materials & Interfaces 15: 10529–10540. DOI: 10.1021/acsami.2c23165.
177. Zhang, P., Guo, R., Zhang, H., et al. (2023). Fluoropolymer coated DNA nanoclews for volumetric visualization of oligonucleotides delivery and near infrared light activated anti-angiogenic oncotherapy. Advanced Science 10: 2304633. DOI: 10.1002/advs.202304633.
178. Yang, L., Zhu, S., He, Z., et al. (2022). Aqueous-phase synthesis of upconversion metal-organic frameworks for ATP-responsive in situ imaging and targeted combinational cancer therapy. Chinese Chemical Letters 33: 314–319. DOI: 10.1016/j.cclet.2021.07.007.
179. Chen, B., Mei, L., Fan, R., et al. (2021). Facile construction of targeted pH-responsive DNA-conjugated gold nanoparticles for synergistic photothermal-chemotherapy. Chinese Chemical Letters 32: 1775–1779. DOI: 10.1016/j.cclet.2020.12.058.
180. Pang, L., Tang, X., Yao, L., et al. (2023). Smart down/upconversion nanomachines integrated with "AND" logic computation and enzyme-free amplification for NIR-II fluorescence-assisted precise and enhanced photodynamic therapy. Chemical

Science **14**: 3070–3075. DOI: 10.1039/D2SC06601G.
181. Zhao, J., Chu, H., Zhao, Y., et al. (2019). A NIR light gated DNA nanodevice for spatiotemporally controlled imaging of microRNA in cells and animals. Journal of the American Chemical Society **141**: 7056–7062. DOI: 10.1021/jacs.9b01931.
182. Zhao, J., Gao, J., Xue, W., et al. (2018). Upconversion luminescence-activated DNA nanodevice for ATP sensing in living cells. Journal of the American Chemical Society **140**: 578–581. DOI: 10.1021/jacs.7b11161.
183. Qu, A., Wu, X., Li, S., et al. (2020). An NIR-responsive DNA-mediated nanotetrahedron enhances the clearance of senescent cells. Advanced Materials **32**: 2000184. DOI: 10.1002/adma.202000184.
184. Jin, Y., Wang, H., Li, X., et al. (2020). Multifunctional DNA polymer-assisted upconversion therapeutic nanoplatform for enhanced photodynamic therapy. ACS Applied Materials & Interfaces **12**: 26832–26841. DOI: 10.1021/acsami.0c03274.
185. Liu, Q., Cheng, H.-B., Ma, R., et al. (2023). A DNA-based nanodevice for near-infrared light-controlled drug release and bioimaging. Nano Today **48**: 101747. DOI: 10.1016/j.nantod.2022.101747.
186. Li, M., Yang, G., Zheng, Y., et al. (2023). NIR/pH-triggered aptamer-functionalized DNA origami nanovehicle for imaging-guided chemo-phototherapy. Journal of Nanobiotechnology **21**: 186. DOI: 10.1186/s12951-023-01953-9.
187. Hu, R., Zhang, X., Zhao, Z., et al. (2014). DNA nanoflowers for multiplexed cellular imaging and traceable targeted drug delivery. Angewandte Chemie International Edition **53**: 5821–5826. DOI: 10.1002/anie.201400323.
188. Szukalski, A., Moffa, M., Camposeo, A., et al. (2019). All-optical switching in dye-doped DNA nanofibers. Journal of Materials Chemistry C **7**: 170–176. DOI: 10.1039/C8TC04677H.
189. Pasternack, R.F., Giannetto, A., Pagano, P., et al. (1991). Self-assembly of porphyrins on nucleic acids and polypeptides. Journal of the American Chemical Society **113**: 7799–7800. DOI: 10.1021/ja00020a072.
190. Hannah, K.C., and Armitage, B.A. (2004). DNA-templated assembly of helical cyanine dye aggregates: A supramolecular chain polymerization. Accounts of Chemical Research **37**: 845–853. DOI: 10.1021/ar030257c.
191. Ahrens, M.J., Sinks, L.E., Rybtchinski, B., et al. (2004). Self-assembly of supramolecular light-harvesting arrays from covalent multi-chromophore perylene-3,4:9,10-bis(dicarboximide) building blocks. Journal of the American Chemical Society **126**: 8284–8294. DOI: 10.1021/ja039820c.
192. Benvin, A.L., Creeger, Y., Fisher, G.W., et al. (2007). Fluorescent DNA nanotags: supramolecular fluorescent labels based on intercalating dye arrays assembled on nanostructured DNA templates. Journal of the American Chemical Society **129**: 2025–2034. DOI: 10.1021/ja066354t.
193. Xu, Y., Huang, S.-w., Ma, Y.-q., et al. (2022). Loading of DOX into a tetrahedral DNA nanostructure: the corner does matter. Nanoscale Advances **4**: 754–760. DOI: 10.1039/D1NA00753J.
194. Li, J., Fan, C., Pei, H., et al. (2013). Smart drug delivery nanocarriers with self-assembled DNA nanostructures. Advanced Materials **25**: 4386–4396. DOI: 10.1002/adma.201300875.
195. Zhang, J., Guo, Y., Ding, F., et al. (2019). A camptothecin-grafted DNA tetrahedron as a precise nanomedicine to inhibit tumor growth. Angewandte Chemie International Edition **58**: 13794–13798. DOI: 10.1002/anie.201907380.
196. Tacar, O., Sriamornsak, P., and Dass, C.R. (2013). Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. Journal of Pharmacy and Pharmacology **65**: 157–170. DOI: 10.1111/j.2042-7158.2012.01567.x.
197. Zhang, Q., Jiang, Q., Li, N., et al. (2014). DNA origami as an in vivo drug delivery vehicle for cancer therapy. ACS Nano **8**: 6633–6643. DOI: 10.1021/nn502058j.
198. Xin, H., Sim, W.J., Namgung, B., et al. (2019). Quantum biological tunnel junction for electron transfer imaging in live cells. Nature Communications **10**: 3245. DOI: 10.1038/s41467-019-11212-x.
199. Xin, H., Namgung, B., and Lee, L.P. (2018). Nanoplasmonic optical antennas for life sciences and medicine. Nature Reviews Materials **3**: 228–243. DOI: 10.1038/s41578-018-0033-8.
200. Gong, P., Lee, C.-Y., Gamble, L.J., et al. (2006). Hybridization behavior of mixed DNA/Alkylthiol monolayers on gold: Characterization by surface plasmon resonance and 32P radiometric assay. Analytical Chemistry **78**: 3326–3334. DOI: 10.1021/ac052138b.
201. Ivanov, A.N., Kuzin, Y.I., and Evtugyn, G.A. (2019). SPR sensor based on polyelectrolyte complexes with DNA inclusion. Sensors and Actuators B: Chemical **281**: 574–581. DOI: 10.1016/j.snb.2018.10.156.
202. Chen, Z., Li, J., Li, T., et al. (2022). A CRISPR/Cas12a-empowered surface plasmon resonance platform for rapid and specific diagnosis of the Omicron variant of SARS-CoV-2. National Science Review **9**: nwac104. DOI: 10.1093/nsr/nwac104.
203. Chen, Y., Chen, Z., Li, T., et al. (2023). Ultrasensitive and specific clustered regularly interspaced short palindromic repeats empowered a plasmonic fiber tip system for amplification-free monkeypox virus detection and genotyping. ACS Nano **17**: 12903–12914. DOI: 10.1021/acsnano.3c05007.
204. Bi, R., Li, Y., Xu, M., et al. (2022). Direct evidence of CRISPR-Cas9-mediated mitochondrial genome editing. The Innovation **3**: 100329. DOI: 10.1016/j.xinn.2022.100329.
205. Tang, D., Jia, T., Luo, Y., et al. (2023). DnaQ mediates directional spacer acquisition in the CRISPR-Cas system by a time-dependent mechanism. The Innovation **4**: 100495. DOI: 10.1016/j.xinn.2023.100495.
206. Zheng, F., Chen, Z., Li, J., et al. (2022). A highly sensitive CRISPR-empowered surface plasmon resonance sensor for diagnosis of inherited diseases with femtomolar-level real-time quantification. Advanced Science **9**: 2105231. DOI: 10.1002/advs.202105231.

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AUTHOR CONTRIBUTIONS

N.N. and O.J. supervised and revised the manuscript. S.X., C.M. and X.X. wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Not applicable.