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An update on advances in new developing DNA conjugation diagnostics and ultra-resolution imaging technologies: Possible applications in medical and biotechnological utilities

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ABSTRACT

DNA molecule engineering has become an attractive discipline in various research scopes. The profound influence of selective and sensitive sensing of DNA molecules in disease diagnosis and molecular imaging is established. In this perspective, we try to shed light on the state-of-the-art technology of DNA bioconjugation assays in DNA biosensor, DNA barcode, DNA nanostructures, and DNA ultra-resolution fluorescence imaging. Non-invasive, simple, and swift biotechniques benefit molecular diagnosis, evaluation of disease stages, and also play a central role in fundamental researches. We discuss the limitations of traditional procedures and the eminence impacts of the advanced methods with clinical applications in timely detection and management of diseases like cancer, genetic disorders, and recognition of microbial pathogens. The predictable and programmable DNA strands have paved the way for cellular and molecular imaging with the ability of single-molecule switching nanoscopy. Consequently, the DNA conjugation tool as an identification paradigm of biological agents in interaction with bio-spesific components is at the heart of biological processes.

1. Introduction

Friedrich Miescher isolated the leukocytes protein in 1869, leading to the identification of high phosphorous and protease-resistant substance, and finally, DNA recognized as a unique cellular component. In 1953, Watson and Crick described the various models of nucleic acid molecular structure and their inventive designs have inspired other scientific studies (Dahm, 2005; Watson, 2011). Scientists across disciplines have advanced DNA application paradigms, particularly in the molecular diagnostics, sensing and imaging. Providing novel molecular techniques may pave the way for practical implementation in genetic disorder detection, screening and analysis during cancer management, monitoring drug responses, identification of bacteria, virus and fungi strains, optical mapping, and high-end imaging of biological processes. Given these aims, the latest bioconjugation methods have improved high-throughput sensing, ultra-high resolution imaging, and exclusive recognition with minimum detection limit, giving significant advantages over conventional methods.

Bioconjugation is an interface between biology and chemistry, implicating covalent linkage of two or more biological and synthetic molecules in which at least one of them is a biomolecule, and can occur between biological moieties such as sugar, oligonucleotide, and other compositions. Some bioconjugated molecules represent a combination of intrinsic functional properties of every single-molecule (Fernandes et al., 2018). DNA bioconjugation is able to hybrid with covalent or non-covalent bonds (Wu et al., 2017), or form chemical modifications on DNA backbone elevating its properties (Kumari et al., 2018), and consequently endow new abilities for molecular diagnostic and imaging (Rashidi et al., 2019). DNA conjugates benefit a variety of processes comprise ultrasensitive recognition of cancer biomarkers (Hu et al., 2010), identification of unknown species (Holmes et al., 2009) investigation of protein DNA interactions (Trads et al., 2017), nucleic acid tracking, single-molecule real-time and live-cell super-resolution imaging (Sun et al., 2017).

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Diverse DNA structures are an outstanding alternative element in hypersensitive detection of miRNA and also have essential roles in upgrading the biosensors selectivity and sensitivity (Hu et al., 2019; Rashidi et al., 2019). Likewise, DNA biosensors can precisely detect various mutations and methylations in a blood sample (Povedano et al., 2018; Zeng and Xiang, 2019). The excellent sensitivity assured by DNA hybridization assay profits DNA barcode probabilities to pinpoint uncharted species, rare alleles and undescribed pathogens. DNA barcoding implicates short oligos that ligate the target genome only when complementary sites are exist, enabling low abundance target analysis (Severins et al., 2018). Antibody-conjugated DNA barcodes allow for multiplex analysis of proteins in single-cell and introduce a novel microfluidic system for viral RNA capturing from blood samples (Du et al., 2017). Furthermore, bio-barcode amplification assay can detect tens of hundreds of biological targets and biomolecules like nucleic acid and protein in the whole sample (Nam et al., 2005).

The DNA molecule can be engineered into a functional nanostructure owing to exceeding predictability and spatial programmability of DNA strands. Self-assembled DNA nanostructures are an increasingly reliable and robust tools for the fabrication and functionalization of DNA strands with macro or nano materials (e.g., polymers, aptamers, and nanoparticles) that can be employed to precisely arrange molecules. The most important and practical DNA nanostructures in molecular diagnosis and fluorescence imaging are DNA origami and DNAzyme (Chen et al., 2018a; Chidchob and Sleiman, 2018; Lu et al., 2017b). DNA origami is a nanoarchitectured material through designing a long DNA single-strand with hundreds of short complementary synthetic single-strand DNA (ssDNA) to detect a kind of small molecule (Lu et al., 2017b). Many small molecules and nanomaterials have been considered to mimic a wide range of enzymes defined as DNA-based catalysts (DNAzymes). Although DNAzymes are old catalysts, they have so far been used to cleavage specific mRNA for down-regulating gene expression. DNAzymes along with other nanostructures can be applied in biosensing and theranostics applications (Morrison et al., 2018; Zhou et al., 2017).

A new DNA-based technology known as DNA points accumulation for imaging in nanoscale topography (DNA-PAINT) is found on probes interaction. In this system, dye-labeled oligonucleotide imager binds to complementary docking strand for improving the spatial resolution of a target (Strauss et al., 2018). Various applications of DNA-PAINT include 3D mapping cellular components and molecules such as proteins on nanoparticles and whole-cell super-resolution imaging (Delcanale et al., 2018; Schueder et al., 2017a). As the imager strands are not fixed on the target, multiplexed images are acquired through imagers solution exchange, an innovation of DNA-PAINT entitled DNA-Exchange PAINT (Lutz et al., 2018). Comparing with other multiplexing approaches like PAINT, Exchange-PAINT give multiple images of targets within one sample in a more straightforward procedure (Agasti et al., 2017). To avoid cross-talk between identical imager strands, the sample is washed after each labeling that causes sample damage. Further efforts lead to FRET-PAINT introduction that overcomes the limitations of Exchange-PAINT, such as background signal (Deußner-Helfmann et al., 2018).

We aim to review accurate and up-to-date molecular diagnostics and fluorescent imaging methods capable of timely detection of high-risk and refractory diseases by their biomarkers, exact biosensing, and exceptional resolution imaging with multiple probes. Moreover, we will emphasize on DNA bioconjugation assays in DNA biosensor, DNA barcode, DNA nanostructure, and DNA-PAINT objects. In addition, we hint the purposes and advantages of each method and section, focused on the strategic role of DNA strands in all of the events.

2. DNA bioconjugation

DNA bioconjugation is a chemical process of covalent or non-covalent linkage used to bind more than 10 base pairs DNA with natural or synthetic moieties including biological molecules (e.g., protein), specific recognition molecules (e.g., aptamer), small molecules (e.g., biotin), synthetic polymers (e.g., N-isopropylacrylamide) or biopolymers (e.g., melanin), drugs (e.g., doxorubicin), fluorescent dyes (e.g., fluorescein), and nanoparticles (e.g., quantum dots). In this regard, DNA molecules are involved in molecular recognition with the primary purpose of providing specificity through selective and high affinity binding (Beals et al., 2019; Kim et al., 2016; Munzar et al., 2018; Shiu et al., 2016; Sun et al., 2016; Trads et al., 2017; Wilks and O'Reilly, 2016; Zhu et al., 2015).

2.1. DNA bioconjugation assays

Generally, the dominant methods used in DNA bioconjugation are hybridization and chemical modification as non-covalent and covalent assays, respectively. DNA hybridization describes complementary base pairing among target and probe oligonucleotides. Competitive DNA hybridization assay is an analysis of the association constant of unlabeled DNA target in the presence of fluorescent label as a tracer. The former technique is applied to next-generation sequencing and DNA microarray with single-molecule fluorescence imaging. Melting temperature (T_m) determines duplex formation stability and hybridization efficiency (Peterson et al., 2016). The 225 different DNA fragments in the genome of *Enterococcus faecalis* and *E. faecium* bacteria recognized by competitive DNA hybridization (Shanks et al., 2006). Decades of subsequent research introduced temperature-robust polymerase chain reaction. In this assay, the competition of designed primer and blocker with 6-14 nt overlap in-between lead to molecular competition on the wild type sequence. Ultimately, the yield of molecular hybridization is about 15% because of synchronic binding of primer and blocker to the same sequence which is energetically undesirable ($\Delta G^{\circ} \sim +2\ \text{kcal mol}^{-1}$). The blocker contains 12-30 nt as a variant enrichment region presenting a mismatch in the template-blocker hybridization with favorable energetic changes ($\Delta G^{\circ} \sim -2\ \text{kcal mol}^{-1}$) and hybridization yield of approximately 95% which is named blocker displacement amplification (BDA). The BDA system is responsible for targeting 9 various genomic regions in the same reaction, selectively amplifying 500 different single-nucleotide variants, using homogenous assays, analyzing clinical cell-free DNA such as cancer mutation detection and monitoring for cancer recurrence with invasive, high-throughput and low-cost tool (Fig. 1a and b) (Wu et al., 2017). Furthermore, hybridization chain reaction (HCR) has been designed as a robust molecular tool and isothermal enzyme-free signal amplification assay. The fundamental scheme of HCR requires DNA hairpin set to derive self-assembly hybridization cascade and a target DNA owning to the initiator sequence to open two DNA hairpins. Eventually, nicked and large double-strand DNA (dsDNA) is formed for bioanalysis, bioimaging, biomedical researches, and clinical diagnosis applications (Bi et al., 2017; Xu et al., 2018). The new generation of HCR ranging from immunosignal hybridization chain reaction (isHCR) and in situ HCR are promising approaches to identify the protein-antigen interaction site in biological samples and single-molecule imaging in combination with highly sensitive multi-cascade assembly. A substantial drawback of antibody-based immunoassays is the low abundance detection of the target molecule which is solved using isHCR assay. is HCR initiators can directly hybridize to any of the different types of DNA HCR amplifiers. This method can yield more than a hundredfold increase in protein recognition sensitivity with the lowest background. Besides, via in situ HCR, each target mRNA is detected using multiple probes each carrying a full HCR. This assay renders automatic background suppression even if reagents bind non-specifically within the samples (Choi et al., 2018; Lin et al., 2018).

Addressing to bioconjugated DNA assay, oligonucleotides chemical modifications are accomplished by combination of 5′-phosphate, ribose sugar, and nucleobase. These groups are capable of reacting with other functional moieties (Khvorova and Watts, 2017). 5′terminal phosphate of DNA strand is extremely reactive, providing an interesting issue for

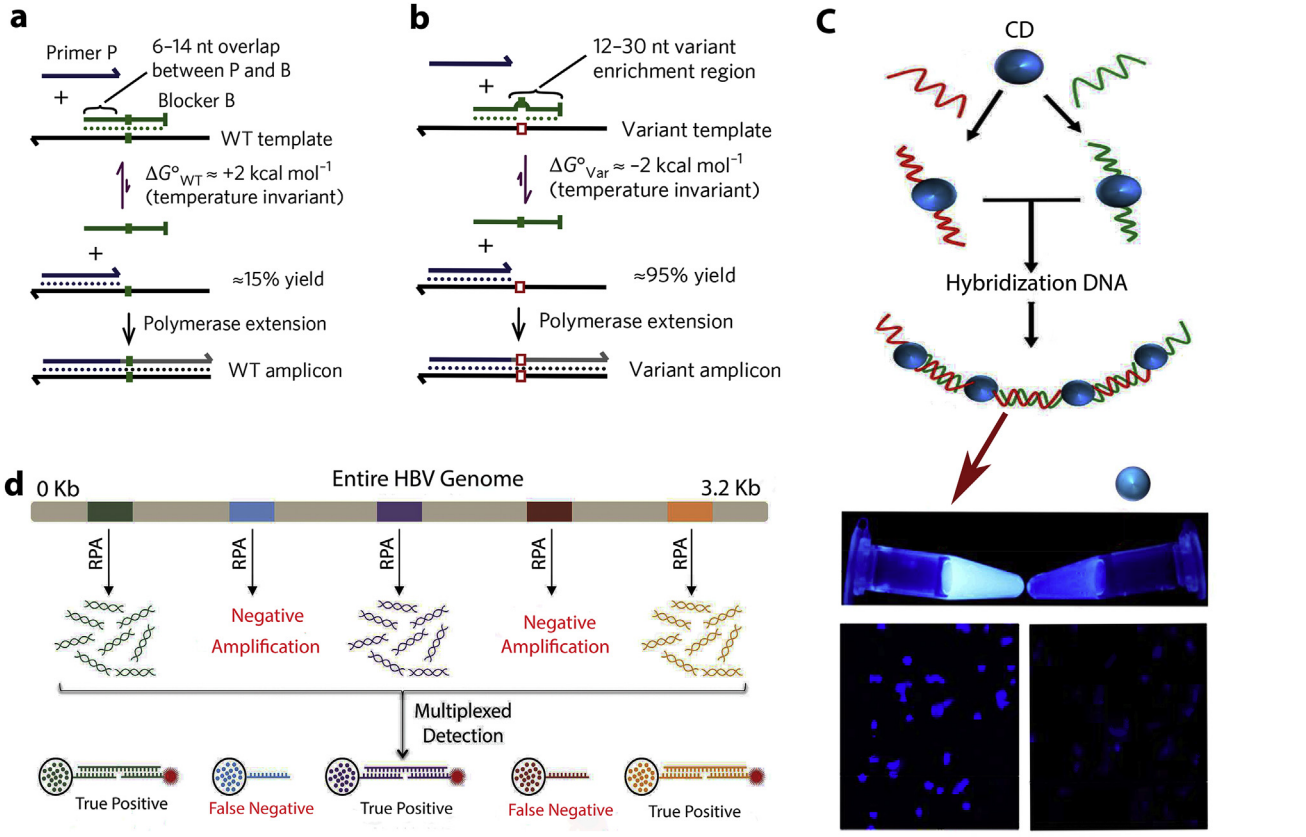

Figure 1. Some diagrams of DNA conjugation assay. **(a)** In BDA system, rare alleles of wild type sequence hybrid with blocker (green) and forward primer (blue) through molecular competition. **(b)** 12–30 nt sequence on the blocker is responsible to form the bubbles in the blocker-template duplex (Wu et al., 2017). **(c)** Linear carbon dot array created through controlled covalent conjugation with DNA (Kumari et al., 2018). **(d)** The amplification of different regions of HBV genome by RPA producing negative and positive products. Template-specific barcodes functionalized with capture DNA molecules are applied to identify denatured amplicons in multiplexed QD barcode assay (Kim et al., 2016).

further modifications. For example, the phosphate attachment to NH_2 functional group of carbon dot enhances the fluorescent intensity of the carbon dot. Several conjugations of ssDNA with carbon dot provides the carbon dot linear array by DNA self-assembly improving cell penetration of carbon dots with bioimaging applications (Fig. 1c) (Kumari et al., 2018). Furthermore, quantum dot (QD) can conjugate to 5′phosphate oligonucleotides, so-called QD barcodes for diagnosing infectious diseases. This goal has been followed by a 3-step development workflow to evaluate from few to full clinical samples. In this study, hepatitis B virus was chosen as the infectious model with mutant ability. Following the extraction of viral DNA from patient serum, various regions of the viral genome are amplified via recombinase polymerase amplification (RPA) to detect viral DNA by multiplex QD barcode method. In the end, fluorescence signals are evaluated by flow cytometry. The main advantages of this method are knows as the remarkable improvement of diagnostic sensitivity up to 25.5%, and multiple target detection (Fig. 1d) (Kim et al., 2016).

Also, amide groups on building blocks, particularly on nucleobases are regular in DNA-conjugates. All aliphatic amides and most aromatic synthetic structures indicate excellent stability to harsh DNA deprotection/cleavage, giving the ability to attach the macromolecules, small peptides, and fluorophores (Yuen and Franzini, 2017). DNA amide linkage with several polymers such as N-isopropylacrylamide is obtained without the need for a catalyst and represents good yield up to 40% in organic solution (Wilks and O'Reilly, 2016). This linkage was exploited between boronic acid and 7-deaza 2′-deoxyadenosine building block as a postsynthetic modification to sense saccharide (Steinmeyer and Wagenknecht, 2018). Bioorthogonal reaction (Devaraj, 2018) on nucleoside triphosphates 2′-sugar have provided unique moiety to conjugate a propargyl functional group on adenosine, uridine, guanosine, and cytidine in the DNA minor groove to form fluorescent probe. This assay has been used in oligonucleotides enzymatic extension and click type ligation with Nile Red azide fluorophore in the presence of Cu(I) (Wenge et al., 2013). Besides, several DNA modification strategies improve selective isolation and targeted DNA plasmid delivery efficiently, i.e. streptavidin-biotin binding assay. One episode of oligonucleotide binds to biotin and another episode bind to an absorbent substance for the purification of specific proteins or template nucleotide sequences placed in the plasmid (Beals et al., 2019; Chen et al., 2017). Also, Biotin-streptavidin coupled with DNA oligonucleotide is applied to human telomerase enzyme activity measurement (Zavari-Nematabad et al., 2017; Alizadeh Ghodsi et al., 2016). In the traditional universal immune-PCR (iPCR) is applied biotin-streptavidin procedure. One limitation of the biotin-streptavidin system is that biotin could be introduced into both of the DNA level and antibody. Toward addressing this challenge, the novel DNA-antibody conjugation methodology upgrade iPCR via covalent binding and nano-structure materials and these conjugants create significant applications in biomolecular ultrasensitive detection (Maerle et al., 2019; Zhang et al., 2017). In a dual coupling of ssDNA probe with biotin and nanoparticles, the detection of pancreatic cancer microRNAs has been conducted at the exosome and residual plasma of the blood samples (Pang et al., 2019). Table 1 summarized the moieties of DNA and target molecules with their varying applications. Each of these conjugation assays reveals DNA bioconjugate importance that choosing the right tool depends on the molecular coupling scheme and the amenability of target. DNA bioconjugation assay designing that works well in a certain application needs a basic understanding of the functionalized

Table 1
Some latest DNA modifications conjugated to target molecules directly (coupling reagents) or by the linker and their main applications.

Functional groups	DNA moiety modification	Target molecule	Linker	application	Ref.
po_4^{2-}-NH_2	5′phosphates	Carbon dot	_	Fluorescent bioimaging Biosensing Theranostics	Kumari et al. (2018)
po_4^{2-} -Zn	5′phosphate	Quantum Dot	_	Infection diagnosis	Kim et al. (2016)
NH_2 -COOH NH_2 -SH	Nucleobase	Polymer	_	Materials scientists	(Wilks and O'Reilly, 2016)
N - COOH	7-deaza-2′-deoxyadenosine	Boronic acid	propylamine	Fluorescent sensing	(Steinmeyer and Wagenknecht, 2018)
OH -N_3	Ribose 2′-O-Propargyl	Fluorescent probe	_	Chemical bioanalytics Fluorescent cell imaging	Wenge et al. (2013)
OH - COOH	3′ OH	Biotin	_	Selective isolation	Chen et al. (2017)
NH_2/COOH OH/COOH	5′phosphate/3′OH	Ag/Biotin	_	Biosensor	Pang et al. (2019)
NH_2 -COOH	3′ NH_2	Antibody	Dibenzocyclooctyne (DBCO)	Biomolecular detection	Maerle et al. (2019)

mechanisms. Ideally, energy variations, temperature, and time are significant factors in oligonucleotides hybridization that affect reaction efficacy. Oligonucleotide modification and hybridization could be ignited a ground-breaking assay in multisite labeling with simultaneous diagnosis and imaging. Given that the undeniable impact of DNA conjugates in bio-detection and bioimaging, photochemical modification of nucleic acid residues in the biocompatible reaction conditions will be improved the bioconjugation quality.

3. DNA biosensor

DNA based biosensor technology has received much attention since the advent of DNA chips (West et al., 2009). Several secondary structures of DNA molecule as a biorecognition element include hairpin DNA (Rashidi et al., 2019), loop stem, random-coil, sandwich (Drummond et al., 2003; Weng et al., 2012), and circuits DNA (Kaufhold et al., 2019). The solid support of biosensors is functionalized by many reagents and materials to attach DNA (Trads et al., 2017). The dsDNA immobilization is a propitious tool in molecular diagnostics with a highly accurate limitation of detection. The adsorped dsDNA can interact with other molecules such as circulating drugs in human blood based on adenine and quinine detectable signals by electrochemical assay (Aydoğdu Tığ et al., 2019). To attain an earlier diagnosis and evaluate the therapeutic efficacy, an anchor-like DNA sensor has raised KRAS G12D point mutation sensing between wild and mutant templates for lung cancer diagnosis on one chip in one step (Zeng and Xiang, 2019). Recently, the possibility of screening type 16 human papillomavirus is provided by the copper oxide nanoparticle with a capturing probe to trap short ssDNA. This identification method is performed with the colorimetric strip that shows superlative reproducibility and stability (Yang et al., 2019). Other nanoparticles like gold nanoparticles (AuNPs) act as a probe by incorporating dsDNA. AuNP conjugated DNA employed for nucleic acid detection is based on competitive oligonucleotide displacement and enhance fluorescence polarization with ultrasensitive identification immediately (Wang et al., 2018). Next survey performed full DNA hybridization by ssDNA probe immobilization on a graphite surface which recognized single nucleotide polymorphism (SNP) with attomolar sensitivity. This biorecognition occurs at the lowest concentration of the target DNA (25 aM) that is confirmed by voltage variation (Campos et al., 2019). To access great efficiency, appropriate selectivity, easy and practical operation for target recognition, the electrochemically controlled reversible addition-fragmentation chain-transfer (eRAFT) polymerization approach is suggested. eRAFT is expected to show more amplified signal in compression to other assays. Peptide nucleic acid oligomer as a hybridization probe is distinguished by eRAFT polymerization, and the detection limit is recognized on the attomolar scale (4.1 aM) in serum samples (Hu et al., 2019). To monitor gene-specific methylation and improve its simplicity and speed, the detection of 5-methylated cytosine (5 mC) is achieved through DNA biorecognition element with no nanomaterial preparation, PCR amplification, and labeling conditions requirement. The methylated DNA is complementary to the biotinylated capturing probe that immobilized on a solid surface. The electrochemical assay has also been implemented on biofluids and cells without previous DNA extraction or amplification with medical applications like cancer development analysis (Povedano et al., 2018). In addition, a multi-functional DNA structure designed with 3 way-junction fragments without loss of function and provide a label-free technique to diagnose pathogens such as avian influenza virus subtype (H5N1, H1N1) in one platform (Fig. 2a) (Lee et al., 2019). Moreover, DNA-protein conjugates produce a highly selective construction to detect target proteins with homologous recognition elements. The steric hindrance coupling would affect the quantitative detection of human antibody and the electrochemiluminescence signal intensity (Liu et al., 2019). To overcome the requirement of costly reagents, elaborated instruments, and target-specific nucleic acid recognition, clustered regularly interspaced short palindromic repeats (CRISPR) system have been introduced. The functionalized graphene as a transistor plate adsorbs deactivated CRISPR-associated protein 9. The detection mechanism relies on the hybridization of a specific single-guide RNA and a target DNA by scanning the genomic DNA sample. CRISPR biosensor is utilized in clinical samples of Duchenne muscular dystrophy (DMD) cases with two deleted mutations in target exons, with the detection duration of 15 min (Fig. 2b) (Hajian et al., 2019). Regarding the recent advancement, recognition of SNPs and transcriptional profiling programs such as gene expression signatures would be promising candidates for gene chip technology.

4. DNA barcode

DNA barcoding is an automatable system for rapid and accurate identification of unknown species by employing short standard regions of DNA as markers instead of using whole-genome (Severins et al., 2018). This approach has proved effective in biodiversity monitoring and authentication of molecular specimens such as various Crustacean taxa (Raupach et al., 2015). Technically, DNA barcoding is a sort of oligonucleotide ligation assay which is a SNP detection and genotyping assay, based on covalent ligation of two adjacent oligonucleotide probes when hybridized to a complementary target sequence (Raclariu et al., 2018). In comparison with prevalent nucleic acid detection methods such as PCR, barcoding require no target pre-amplification which is a time consuming, an expensive and probably error-prone step that may consequently lead to false-positive results (Du et al., 2017; Potapov and Ong, 2017). Barcoding was first coined by Herbet and is provided by electrostatic or hydrophobic interactions (Hebert et al., 2003). The process investigates variable nucleotides existing among standard regions of the genome, which are key informative features for the classification of species. For example, cytochrome oxidase 1 (COX I)

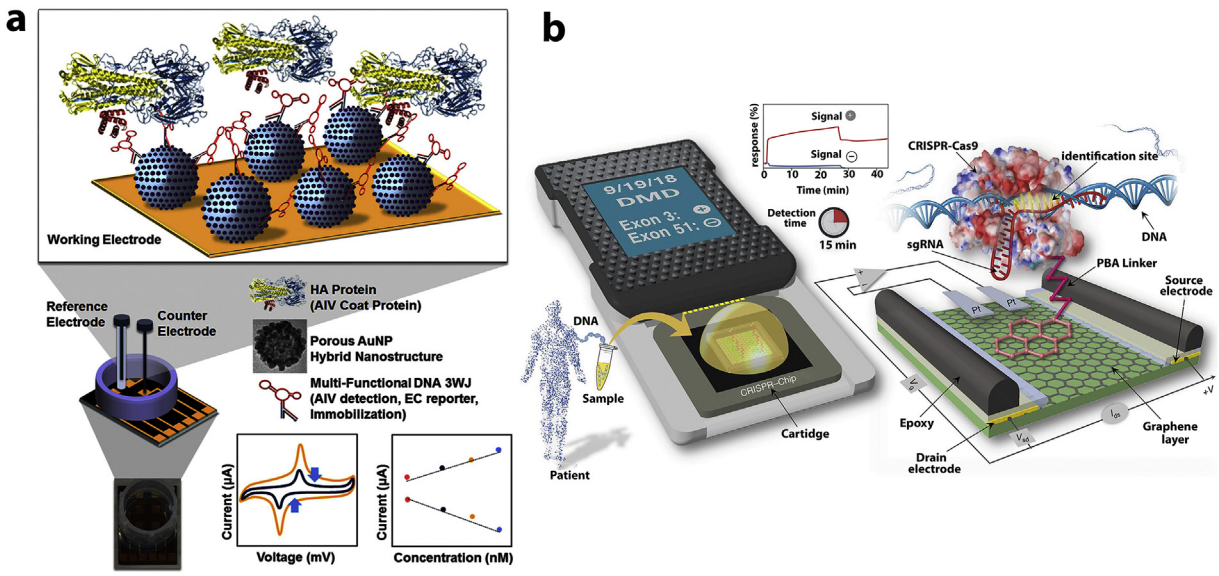

Figure 2. Schematic images of DNA conjugation biosensors. **(a)** The manufactured AIV detection biosensor (Lee et al., 2019). **(b)** DMD disorders gene deletion detected by CRISPR-chip (Hajian et al., 2019).

presented in the mitochondrial gene, is an index used in animal species identification but its desired variability in plant genome is dramatically low (Bucklin et al., 2011). Chloroplast gene ribulose1,5,bisphosphate, mat K, and trnHpsbA are three common DNA barcodes used for plants identification (Kress, 2017; Moon et al., 2016). Internal transcribed spacer (ITS) is a reliable marker for fungi identification as Cunninghamella species in clinical samples. Cunninghamella leads to mucormycosis, which is a severe infection with high mortality in patients with impaired immune system (Yu et al., 2014). Barcodes are commonly labeled with spectrally separated fluorophores enable them to be detected by fluorescence spectrophotometry. The information is referred to Gene Bank to locate the investigated samples in phylogenetic categories or to create new genetic sequence libraries (Kress, 2017). Bio-barcode approach with the benefit of nanoparticles such as AuNP breeds a novel generation of biosensors in the accurate and rapid detection of toxins in foodstuff such as enterotoxin B and exotoxin A (Amini et al., 2017; Xu et al., 2019). Bio-barcode acts as a signal reporter probe that specifically binds to the target DNA in companion with the second DNA probe, which is coated with nanoparticle and binds the other end of the target (Amini et al., 2017). The possibility of investigation in human genetic diversity and barcoding of new generation sequencing (NGS) libraries for ultrasensitive detection of mutations is provided through DNA barcoding. Detection of remarkably rare alleles within an obscurant DNA mixture such as circulating tumor DNA in cancerous plasma is of increasing interest in many basic and clinical studies (Ståhlberg et al., 2017). One prevalent obstacle of NGS is the background noise that keeps the detection of very rare single nucleotide variants still challenging (Fox et al., 2014). The main source of this background noise is DNA polymerase errors occur while library construction or sequencing. Approaches to solve this problem are usually based on the combination of deep sequencing, bioinformatics and barcoding of DNA templates or even each of them individually (Kennedy et al., 2014). DNA barcoding in NGS proffers a way for early recognition of rare alleles and bioinformatically removes polymerase errors which ultimately solves the background noise. However, barcoding is not capable of correcting some sequencing errors which are not induced by polymerase such as chemically modified bases (Ståhlberg et al., 2017). Chromatin immunoprecipitation accompanied by deep sequencing (ChIP-seq) is a valuable technique in epigenetics with biological and clinical applications. The process involves ligation of barcodes to identify signals from sample, immunoprecipitation, and purifying the barcoded DNA into a sequence library. An innovation of barcode ChIP-seq, named restriction enzyme-based labeling of chromatin in situ provides multiplex sampling in a single ChIP leading to dramatically high-throughput results (Arrigoni et al., 2018). Recently, there has been a great interest in developing a dynamic and strong barcoding system to provide reliable identification capabilities (Yu et al., 2017). DNA meta-barcoding as a special case is applied for recognition of samples with more than one organism and employs the same database as barcoding resulted in high-throughput sequencing of taxa from mixed samples (Kimmerling et al., 2018). DNA barcoding become an indispensable tool in the assessment of modern biodiversity and play an important role in most of the conservative biological disciplines.

5. DNA nanostructure

The landmark idea of DNA nanotechnology is that the DNA molecule takes out of its heritable biological background and uses DNA base pairing to assemble structural motifs to produce diverse two-dimensional (2D) and three-dimensional (3D) DNA crystals. With respect to specific base pairing, self-assembled DNA nanostructures provide acquisitive properties with different applications including molecular diagnosis and theranostics, drug delivery, biosensing, fundamental research in biomedical and material sciences, and manufacture of multipart structures. DNA nanostructures exhibit good biocompatibility, small size, desired secondary structures, and high programmability (Chen et al., 2018b; Chidchob and Sleiman, 2018). DNA nanostructures serve an efficient platform for binding aptamers (Lu et al., 2017b), oligonucleotide probes (Choi et al., 2019), enzymes (Sun et al., 2017) polymers (Jia et al., 2019), and other biomolecules (Wei et al., 2016). In this section, the major focus is on DNA nanostructures with molecular diagnosis and imaging applications, including DNA origami and DNAzyme.

5.1. DNA origami

DNA origami forms by programmable self-assembled nanofabrication, arbitrarily shaped structures with a bottom-up approach that have tremendous potential to function as versatile platforms with controlled stoichiometry. The DNA origami fabrication typically consist of several steps: designing long ssDNA scaffold isolated from virus genome,

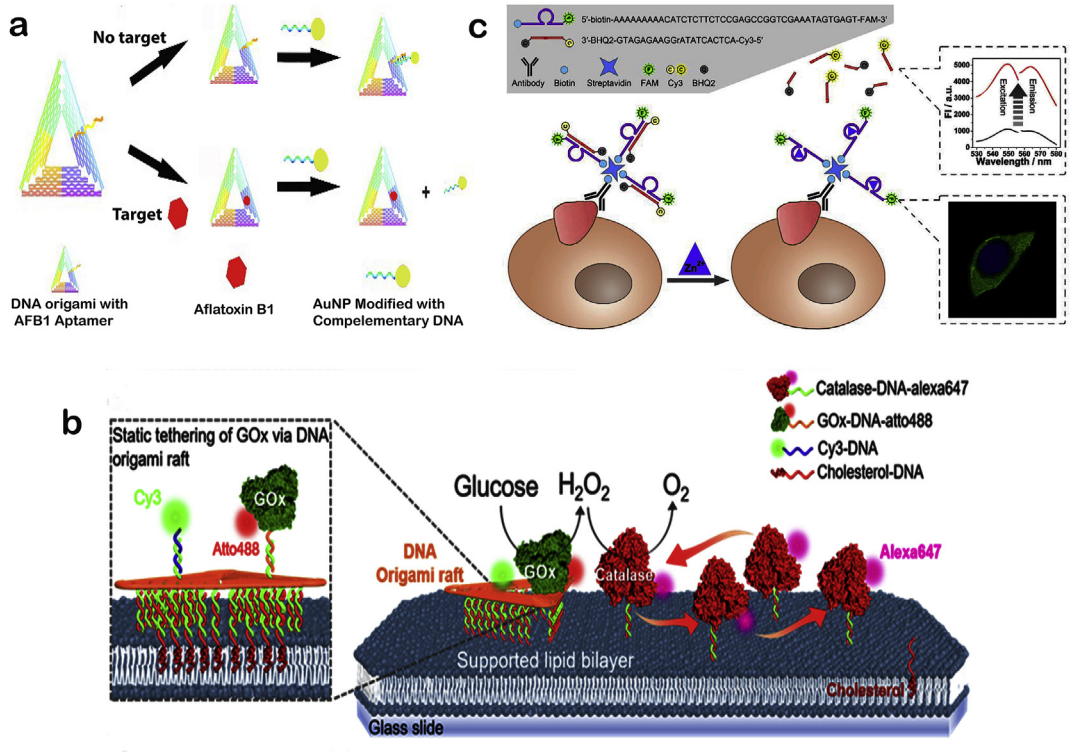

Figure 3. DNA nanostructure application in molecular diagnostics and imaging. **(a)** The aptamer-tagged DNA origami for AFB1 detection (Lu et al., 2017b). **(b)** Single-molecular enzyme cascade real-time imaging via DNA origami (Sun et al., 2017). **(c)** Tumor-associated membrane protein integrating imaging by DNAzyme based nondestructive (Chen et al., 2018b).

mixing of hundred synthetic ssDNA staples strands, formation of 2D or 3D predesigned shapes, computer designing of DNA origami, ssDNA extension and protein or nanoparticle modification, DNA sequencing, and characterization by transmission electron microscopy (TEM) and atomic force microscopy (AFM) (Kuzyk et al., 2018). The precise molecular design of DNA origami simplifies handling biomolecules (Farimani et al., 2017) and detection of small molecules like aptamer. An interesting example of DNA origami interactions is aptamer-tagged DNA origami with AuNPs conjugated oligonucleotides that are paired with aptamer in order to detect aflatoxin B1. The amount of traceable aflatoxin B1 is associated with AuNPs attachments with the specific sites of designed DNA origami. In the presence of aflatoxin B1, the Au-oligonucleotide is not able to anchor on DNA origami substrates, and ultimately, the quantity of AuNP-DNA origami complex is decreased (Fig. 3a) (Lu et al., 2017b). In the same study, to prevail the drug resistance and high mortality of malaria disease, malaria biomarker was selected through programmable molecular technology. *Plasmodium falciparum* specific aptamer-tagged DNA origami is able to bind lactate dehydrogenase enzyme, and this interaction is recognized by high speed AFM. The supramolecular DNA scaffold integration opened a potential window to early, sensitive, and accurate diagnosis of the malaria biomarker (Godonoga et al., 2016). On the other hand, DNA origami plays a pivotal role in single-molecule detection (Puchkova et al., 2015) and brighter fluorescence imaging with enhanced fluorescence intensity. DNA origami engineering is a sensitive technique for fluorescence-based detection like microbead-based assay. Functioned microbeads by streptavidin are conjugated to biotinylated capturing DNA able to hybridization with target oligonucleotides such as miRNA. In this line, simultaneous attendance of multiple dyes and various strands in two different trapezoid of the triangular DNA origami are important for making diverse probes to enhance the signal and binding strength of the target oligonucleotide with reporter probe. Finally, the sensing event measured by VideoScan technology. Compared with conventional single dye and single strand reporters, DNA origami is more efficient candidate for quantification of the target oligonucleotides at low concentrations (Choi et al., 2019). Raft-based DNA origami is a practical structure for in situ real-time single-molecule tracking of enzyme motion. Functionalized origami could incorporate with lipid bilayer cholesterol allowing Glucose oxidase and catalase enzymes to anchor on cholesterol-labeled origami rafts and dsDNA, respectively. The motion and cascade of enzymes visualized by the total internal reflection fluorescence microscopy (TIRFM). This method is developed to investigate the dynamic interaction of protein and high resolution imaging of the individual enzyme motion on a 2D fluidic surface (Fig. 3b) (Sun et al., 2017). With respect to programmable DNA molecule, predictable base pairing and nanoscale engineering of this molecule, cellular and molecular following would be achievable by overcoming the high resolution imaging obstacle.

5.2. DNAzyme

The ability of ssDNA in folding to 3D structure can mimic enzymatic function. This event requires displaying of so-called DNA-based enzyme, deoxyribozyme or DNAzyme to cleavage a specific RNA sequence and catalyze chemical transformations. DNAzymes have not been found naturally, but are synthesized chemically via a combinatorial biology technique known as *in vitro* selection. In addition to the target RNA cleavage, other chemical activities are performed by DNAzymes ranging from DNA phosphorylation, DNA cleavage, and DNA/RNA ligation. These bio-inspired molecules are sensitive to a broad range of analytes. The DNAzymes salient features such as tailored stability and programmability have made them a suitable choice for bioanalytical assay, biosensor designing, and theranostic applications. The specific cleavage of RNA toward DNAzymes performs a crucial role in sensing and early detection of viral and cancerous diseases (Morrison et al., 2018; Zhou et al., 2017). One of the hottest topics of DNAzymes is DNA biomarker detection at ultra-low concentrations. A colorimetric strategy based on multi-amplification nanofibrous sensing is suggested

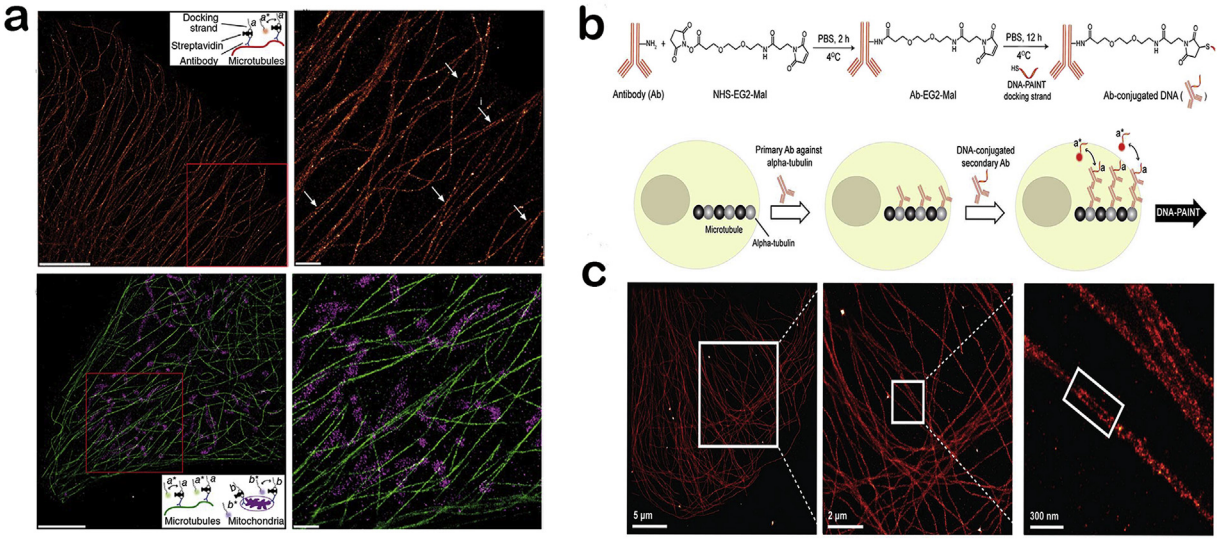

Figure 4. Super-resolution fluorescent imaging. **(a)** Multiplexed DNA-PAINT imaging of microtubules and mitochondria imaging inside a fixed cell, magnified in view of the boxed area in the left photos (Jungmann et al., 2014). **(b)** Labeling scheme of antibody-DNA conjugation imaging. **(c)** Secondary antibody-based DNA-PAINT imaging of microtubules inside a fixed cell. Microtubule structure clearly illustrate different magnifications: 5 µm, 2 µm, and 300 nm (Agasti et al., 2017).

for ultrasensitive visual detection of HIV DNA in blood samples. Given that indicated assay combines the catalytic hairpin assembly amplification reaction, the DNAzyme-catalyzed colorimetric reaction, and glucose oxidase as a biocatalyst. This method is facile and label free with nanomolar sensitivity to distinguish single-base mismatch (Long et al., 2016). In another colorimetric method to detect 3 strains of Classic Swine Fever Virus, nucleic acid sequence-based amplification and G-quadruplex DNAzyme assay were used (Lu et al., 2017a). Recently, tremendous efforts are directed to overcome the limitations of conventional methods including flow cytometry, real-time quantitative PCR, fluorescence in situ hybridization, chromosome analysis such as low sensitivity, technical complexity, and costly equipment to improve gene deletion diagnosis by novel strategies. G-quadruplex DNAzyme assay driven chemiluminescence imaging has been developed for detection BCR/ABL fusion gene based on bis-three-way junction (bis-3WJ) nanostructure and DNA cascade machinery. Bis-3WJ probe recognizes BCR/ABL fusion gene through the creation of a sustainable bis-3WJ nanostructure and then activated polymerase/nicking enzyme machineries in cascade to synthesis the DNAzyme subunits. These subunits are integrated with DNAzyme by self-assembly to catalyze chemiluminescence substrate and eventually generate the signal. This method is amenable to diagnose chronic myelogenous leukemia with low detection limit of less than 23 fM (Xu et al., 2016). Besides, colorimetric assay based on AuNPs is used in visual detection of pathologic proteins that require proximity ligation and DNAzyme to hydride the probes and rise the specificity of the reaction (Wei et al., 2016). The novel and simple method for both quantification of tumor-associated membrane proteins (TMPs) and in situ imaging are introduced by providing the dual-labeled DNAzyme based on fluorophore imaging. Dual-labeled DNAzyme is modified via biotin/FAM fluorophore and Cy3 fluorophore/BHQ2 quencher, respectively. Biotin-modified DNAzyme is hybridized to TMPs biotinylated antibodies via streptavidin-biotin system on the cell surface. In the presence of zinc ion, ion dependent DNAzyme releases the substrate DNAzyme strand. The expression level of TMPs is evaluated by increased fluorescence signal of the ion dependent DNAzyme and its catalytic activity (Fig. 3c) (Chen et al., 2018b). Collectively, the new progress considerably improves simultaneous detection and cleavage of target mRNA and is a propelling DNAzyme based tool with theranostics applications.

6. DNA PAINT

Recent advances in super-resolution microscopy have revolutionized the concept of conventional microscopy by eliminating the classical diffraction limit of light, allowing researchers to investigate subcellular structures and procedures in further details (Agasti et al., 2017). One convenient way of visualizing biological processes and molecular ensembles by super-resolution imaging is PAINT (Schnitzbauer et al., 2017). This technique is placed in the category of single molecule localization microscopy (Beliveau et al., 2017) and relies on transient conjugation of fluorescently labeled probes with complementary targets which are diffused freely in an image buffer (Kiuchi et al., 2015). DNA-PAINT is capable of super-resolution imaging or so-called nanoscopy of a large variety of targets such as cellular proteins, DNA and RNA and other cellular structures with up to 80 µm axial and 20 µm planar resolution, and does not require any dye photoswitching and target modifying (Dai et al., 2016; Schueder et al., 2017a). The latest innovations of PAINT which overcome the limitation of multiplexing capacity in super-resolution imaging are Exchange PAINT, Förster resonance energy transfer PAINT (FRET-PAINT), quantitative PAINT (Jungmann et al., 2016; Nieves et al., 2018). The techniques allow for 3D multiplexing of molecular components like mitochondrial proteins (HSP90), EGFR receptors (LIMP-2), nuclear architectural proteins (Lamin B) and plenty of other cellular targets (Fig. 4a) (Jungmann et al., 2014; Silverberg et al., 2015; Zheng et al., 2018). This method permits highly sensitive multiplexing with the application of primary DNA structures such as 2D and 3D aptamers and somamers, streptavidin and secondary antibody conjugated DNA probes in multiple colors (Delcanale et al., 2018; Opazo et al., 2012). DNA-PAINT is based on either transient hybridization of short oligonucleotides named docking and imager strands or their statically interaction, which produce blinking patterns for super-resolution imaging (Opazo et al., 2012). Docking strand is a short DNA oligonucleotide conjugated on molecular target of interest and has a complementary sequence to interact with the imager strand, which is labeled with a fluorophore (Schnitzbauer et al., 2017). DNA-PAINT efficiency is affected by the concentration of the imager strand and its binding strength to docking strand (Jungmann et al., 2010). The transient and stochastic binding of the mentioned strands is due to hydrophobic or electrostatic interactions, leading to switching ability between ON and OFF state (Fig. 4b and c) (Agasti et al., 2017). Upon binding the imager strand (ON state), fluorescence emission is detected and consequently

analyzed using highly inclined and laminated optical sheet (HILO) or total internal reflection fluorescence microscopy (TIRF) (Jungmann et al., 2010; Tokunaga et al., 2008). One extension of the DNA-PAINT is DNA-Exchange PAINT which provides a novel multiplexing approach in super-resolution microscopy along with circumventing some of DNA-PAINT limitations such as delayed image acquisition or non-fluorogenic imager strand employment (Jungmann et al., 2016). The Exchange-PAINT probes are shorter in comparison with DNA-PAINT probes and permit rapid exchange in sequential imaging round, but there may be unoccupied docking strands too (Nieves et al., 2018). Briefly, Exchange-PAINT is based on the ability of manipulating docking strand with specificity for imager strand. Repeated imaging cycles with different complementary imaging strands require critical washing step after each imaging cycle (Werbin et al., 2017). The washing step prevents any cross-talk between two distinctive imager strands, but the probability sample distortion through washing steps, especially in feeble tissues, should be considered. Washing buffer contains denaturation mediators such as formamide (Schueder et al., 2017b). The described method enables visualization of multifarious cellular structures such as microtubules and mitochondria in fixed cells in high resolution through immunostaining by means of anti-body conjugated DNA strands (Jungmann et al., 2014; Wang et al., 2017). Super-resolution imaging of mitochondria and microtubules in the presence of HSP-60, by exchange-paint have led to whole-cell imaging. The nanoscopy of molecular components such as RNA and DNA in fixed cells is acquirable through FISH-type experiments (Beliveau et al., 2017; Schueder et al., 2017a). Exchange-PAINT has proven to be an effective tool in super-resolution microscopy, but according to some limitations of the technique, ranging from unspecific interactions between the imager strands and off-target sites (Auer et al., 2017). Moreover, background fluorescence, low sample penetration depth, lengthy washing steps and a demand for an intricate fluidic system still are challenging subjects about Exchange-PAINT (Lee et al., 2017; Zhao et al., 2015). To overcome these limitations several approaches have been developed. FRET-PAINT and Quencher-Exchange-PAINT could be counted as illustrations. FRET-PAINT is a combination of DNA-PAINT with single-molecule FRET (smFRET) which provides super resolved images with precise distance readout near to molecular range from 1 to 10 nm. This technique, instead of one, uses pairs of DNA oligos as imager strands and one docking strand that is conjugated to specific parts of the target. One of the imager strands is labeled with a donor fluorophore while the other one carries an acceptor dye (Deußner-Helfmann et al., 2018). Different docking strands have to be an inappropriate distance of each other in a way to meet two aims. The mentioned distance has to be small as possible to require sub-diffraction limit of light microscopy and on the other hand it has to be large enough to prevent FRET between docking strands (Szendi-Szatmári et al., 2019). The fluorescence signal is resulted from FRET occurrence due to simultaneous binding of both complementary imager strands to the same docking one. The application of the high concentration imager strands leads to faster image acquisition and alleviates the false positive and unspecific signals from single imaging strand binding to docking strand (Auer et al., 2017). There are two different approaches for FRET-PAINT performed through either fixed or transient binding of the acceptor dye labeled DNA strand with transiently donor dye labeled one. In the fixed acceptor version, the fastest readout is feasible due to the permanent availability of the acceptor dye, but it cannot be replenished and so this version is disposed to photobleaching of the acceptor. The former limitation is resolved by dynamic FRET-PAINT where both of the donor and acceptor strands interact with the docking strand transiently (Lee et al., 2017). Fluorescence emission of the acceptor dye is detected by a dual channel single-molecule microscope, which employs TIRF illumination (Ellefsen et al., 2015). In comparison with conventional DNA-PAINT, FRET-PINT profits some advantages like minimized background signal. Another advantage of FRET-PAINT is the increased speed of image acquisition by 8–30 folds in comparison with DNA-PAINT due to optimizing the dissociation rate of DNA probes (Lee et al., 2017). Recently developed FRET-PAINT is an alternative technique of DNA-PAINT with no photobleaching problem and facilitates the study of dynamic processes, with a wide range of applications such as visualizing membrane bound or cell surface molecules of living cell and the investigation of protein arrangements and mRNA patterns in nano-scale (Jungmann et al., 2016, 2014). To date, FRET-PAINT has provided super-resolution 3D images of various samples such as neural tissue samples, Cos-7 microtubules and *Hella* cell sub-cellular structures (Lee et al., 2017). It is envisioned that FRET-PAINT would be capable of sub-diffraction resolved monitoring of protein conformations while detecting its localization at the same time.

7. Conclusions

In this article, we reviewed DNA bioconjugation importance that is useful in obtaining highly accurate molecular diagnostics and super-resolution cellular and molecular imaging into DNA biosensor, DNA barcode, DNA nanostructure, and DNA-PAINT issues. Further, the novel reliable mythologies in accordance with DNA conjugation procedures for the determination of cancer biomarkers, infectious diseases, and genetic disorders are discussed.

Nowadays, the main challenges are detection of both primary and metastatic tumors, recurrent cancer cases, circulating tumor DNA (exosome, miRNA), response to treatment, and molecular alterations in neoplastic cells an early stage that need to precise diagnosis and clinical prognosis as well as screening and patient monitoring. Moreover, DNA sequence aberrations such as SNPs, point mutations, exon deletions, rare allele detection and their variations copy number, epigenetic alternations; in particular DNA methylations have proven to be a key parameter of molecular diagnostic and bioimaging. The aforementioned challenges entail innovative molecular assays with high sensitivity, timesaving, label free, economical, multiplex real-time imaging, and without need to target pre-amplification to overcome the limitations and disadvantages of conventional methods ranging from PCR, fluorescence in situ hybridization, and flow cytometry.

This concept allows the use of DNA strands to conjugate biological or synthetic materials. Naked or modified DNA strands can be used as probes on the recognition element of DNA biosensor, DNA barcodes, an oligonucleotide with specific chemical reaction property and multi-dimensional constituent units in DNA nanostructures, and finally as imager and docking strands in DNA-PAINT.

8. Future perspectives

The hybridization of complementary DNA sequences and their chemical modifications are central tenets of nucleic acid chemistry that make DNA an individual tool in biomedicine. Regarding regeneration and high sensitivity of the recognition layer of DNA biosensors, commercial production of these portable devices with biomedical applications are anticipated. Addressing to DNA barcode technology, mitochondrial DNA along with nuclear DNA would be applied in forensic palynology in futuristic schemes, conclusively. DNA is highly programmable and applicable to build functional nanostructures in point-of-care molecular diagnostics. Single-molecular fluorescence imaging in the ultra-violet wavelength range would be used as an accurate whole-body imaging system by replacing fluorescence materials in the near infrared wavelength range with increasing penetration depth. Although late to the suite of current clinical molecular diagnostics of DNA conjugation technologies, it is a promising diagnostic method for early identification of special nervous or mental diseases and quantitative imaging during ongoing follow up and clinical translation.

CRediT authorship contribution statement

Nasrin Mohajeri: Writing - review & editing, Investigation,

Software. **Mahsa Imani:** Writing - review & editing, Investigation, Resources. **Abolfazl Akbarzadeh:** Conceptualization, Supervision. **Alireza Sadighi:** Software, Resources. **Nosratollah Zarghami:** Supervision, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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