

Review

Recent Advances in DNA Systems for In Situ Telomerase Activity Detection and Imaging

Shiyi Zhang ^{1,2,3,4} , Wenjing Xiong ^{1,2,3,4}, Shuyue Xu ^{1,2,3,4} and Ruocan Qian ^{1,2,3,4,*} 
¹ Key Laboratory for Advanced Materials, East China University of Science and Technology, Shanghai 200237, China; zhangshiyi@mail.ecust.edu.cn (S.Z.); 21010766@mail.ecust.edu.cn (W.X.); xushuyue@mail.ecust.edu.cn (S.X.)

² Feringa Nobel Prize Scientist Joint Research Center, Joint International Laboratory for Precision Chemistry, East China University of Science and Technology, Shanghai 200237, China

³ Frontiers Science Center for Materiobiology & Dynamic Chemistry, East China University of Science and Technology, Shanghai 200237, China

⁴ School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China

* Correspondence: ruocanqian@ecust.edu.cn

Abstract: Telomeres play a key role in maintaining chromosome stability and cellular aging. They consist of repetitive DNA sequences that protect chromosome ends and regulate cell division. Telomerase is a reverse transcriptase enzyme counteracts the natural shortening of telomeres during cell division by extending them. Its activity is pivotal in stem cells and cancer cells but absent in most normal somatic cells. Recent advances in biosensor technologies have facilitated the in situ detection of telomerase activity, which is essential for understanding its role in aging and cancer. Techniques such as fluorescence, electrochemistry, and DNA nanotechnology are now being employed to monitor telomerase activity in living cells, providing real-time insights into cellular processes. DNA-based biosensors, especially those incorporating molecular beacons, DNA walkers, and logic gates, have shown promise for enhancing sensitivity and specificity in telomerase imaging. These approaches also facilitate the simultaneous analysis of related cellular pathways, offering potential applications in early cancer detection and precision therapies. This review explores recent developments in intracellular telomerase imaging, highlighting innovative approaches such as DNA-functionalized nanoparticles and multi-channel logic systems, which offer non-invasive, real-time detection of telomerase activity in complex cellular environments.

Keywords: telomerase activity; single-cell imaging; DNA probe



Received: 8 December 2024

Revised: 13 January 2025

Accepted: 13 January 2025

Published: 15 January 2025

Citation: Zhang, S.; Xiong, W.; Xu, S.; Qian, R. Recent Advances in DNA Systems for In Situ Telomerase Activity Detection and Imaging. *Chemosensors* **2025**, *13*, 17. <https://doi.org/10.3390/chemosensors13010017>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The concept of telomeres dates back to the 1930s, when geneticists Barbara McClintock and Hermann J. Muller first proposed the idea [1]. In the 1980s, with the determination of the human telomere sequence, researchers uncovered the functions of telomeres and telomerase, establishing a connection between telomeres and cellular aging [2,3]. Human telomere DNA is a repetitive sequence of TTAGGG approximately 5–15 kb in length at the end of chromosomes [4]. The telomere has a DNA architecture comprising a double-stranded DNA (dsDNA) region several kilobases long and ending with a single-stranded 3' tail known as the G-overhang [5]. In mammals, telomeric DNA appears regularly at the ends of chromosomes and is highly polymorphic within the population, which is characteristic of periodic sequences [6–9]. The DNA sequence of telomere repeats can interact with

the Shelterin protein complex, which consists of six telomere-binding proteins, ultimately forming a lock-like structure (t-loop) that further protects the telomere end [10,11]. Telomere length is highly heterogeneous, even within a single cell [12]. During cell proliferation, telomeres gradually shorten due to events such as delayed chain replication, oxidative damage, nucleic acid excision, and other processes [13,14]. When telomere length shortens to a certain level, cell proliferation disrupts normal DNA segments, and these lesions accumulate to a point that leads to apoptosis [15,16]. Therefore, telomere length maintenance plays an important role in cell biology, and its deregulation can lead to premature aging and cancer [17].

Telomerase was first cloned in 1997 [18]. It is a reverse transcriptase enzyme that is made of Ribonucleoprotein and can use its own RNA as a template to extend telomere DNA [19]. Human telomerase consists of non-coding human telomerase RNA (hTR, also called hTER or hTERC) and human telomerase reverse transcriptase (hTERT) [20,21]. Commonly, immortalized cells and cancer cells usually have high telomerase activity [22]. TERT is rarely detected in normal human cells, except in embryonic, germ, and stem cells [23].

For the majority, hTR contains a total of 451 nucleotides, and longer forms of this transcript have been reported [24,25]. It tethers the telomerase and includes two distinct functional lobes: a catalytic core and a Hinge and ACA (H/ACA) box RNP [26]. In the catalytic core, the pseudoknot/core domain is essential for telomerase activity and containing the template sequence [27]. Additionally, conserved regions 4 and 5 (CR4/CR5) are crucial for TERT association [28,29]. During telomere synthesis, the non-template part of hTR plays an important role, including translocation between nucleus and cytoplasm, for example, a Cajal body localization factor, TCAB1 [30]. The primary function of hTR is to provide the RNA template for DNA synthesis [31]. Telomerase utilizes this intrinsic template to synthesize telomeric DNA repeats, adding new telomere sequences during each cell cycle [32]. In normal cells, hTR is commonly expressed as lncRNA [33]. But it should be noted that previous studies revealed that hTR levels do not always correlate with detected telomerase activity, as some tumors express telomerase RNA but lack telomerase activity [34].

hTERT, consisting of 1132 amino acids, contains four telomerase-specific motifs: the telomerase essential N-terminal (TEN) domain, the TERT RNA-binding domain (TRBD), the reverse transcriptase (RT) domain, and the C-terminal extension (CTE) [35,36]. The process of telomere elongation involves multiple proteins and precise regulation [37]. The TEN domains of TERT serve as an anchor for binding to telomeric DNA, initiating the primer–template interaction required for telomere elongation. The CTE and TRBD domains simultaneously interact with the CR4/5 domain of hTR [38,39]. This interaction ensures proper positioning of the TERT domain after TPP1 (the component of Shelterin) binds to the TEN domain, allowing the anchor site to engage the DNA substrate [27]. However, the composition of the telomerase protein varies greatly between species [40,41]. For example, *Caenorhabditis elegans*, *Caenorhabditis briggsae*, and *Caenorhabditis remanei* seem to be missing this structure [42].

Biosensors play an important role in early diagnosis of diseases. A large number of studies have been developed to characterize and quantify telomerase activity, including the polymerase chain reaction (PCR)-based telomeric repeat amplification (TRAP) protocol [43] and PCR-free methods, such as colorimetry [44], fluorescence [45], electrochemistry [46,47], chemiluminescence [48], enzyme-linked immunosorbent assay (ELISA) [49,50], dark-field microscopy (DFM) [51], and so on. Polymerase chain reaction (PCR) and isothermal DNA amplification require large amounts of a cell and cell lysis, resulting in the loss of cells and intracellular targets [52]. However, due to the difficulty of probe entry into cells

and interference from complex environments, most applications focus on the detection of telomerase extracted from lysis, urine samples, or tissue samples [53]. These samples often contain high concentrations of proteins or other complex matrices, which can lead to false results [54,55]. Immobilization of cells requires complex pre-processing steps, including formamide, T4 DNA ligase, and DNA ligase and RNase inhibitor, and does not respond to the physiological activity of the cells in real time. Therefore, in situ detection of telomerase activity remains essential for understanding its critical role in physiological processes. DNA is a highly stable polymer that is easy to synthesize and program, has a long shelf life, and offers low production costs, making it an ideal building block for the assembly of nanomaterials and targeting biomolecules [56,57]. Recently, various advanced methods based on DNA sequences have been developed for the direct imaging of intracellular telomerase activity in living cells. These methods offer a non-invasive, real-time approach to better understand telomerase-related carcinogenesis and accurately evaluate and guide anticancer treatments. Currently, in situ imaging techniques primarily detect two types of targets, single-stranded DNA binding to the hTR template and double-stranded DNA resulting from the extension of the telomerase template, both serving as markers of telomerase protein. This review summarizes recent advancements in intracellular telomerase in situ imaging based on DNA nanotechnology, including associated telomere elongation and repair pathways, as well as key proteins involved.

2. Fluorescence-Based Imaging and Nanomaterial-Assisted Delivery for In Situ Telomerase Activity Detection

Fluorescence imaging has emerged as one of the most popular imaging methods because of its high sensitivity, easy operation, and good repeatability [58]. Nucleic acid probes that feature fluorescent signals hold great promise for detecting biomolecular interactions because of their high affinity for target sequences and minimal interference with other components [59]. One notable application is the use of fluorescent RNA probes for cell imaging, as demonstrated by Li et al. They developed a spatially confined fluorescent RNA probe for in situ imaging, generated through cascaded rolling circle transcription and DNA fixation [60]. In the presence of hTR, P1 was circularized and used as a template for T7 RNA polymerase, generating long RNA that opened HP regions. The unfolded HP hybridized with P2, producing spinach RNA aptamers, which, upon DFHBI insertion, generated an enhanced fluorescent signal. Due to the rigid steric hindrance, the probe showed good nuclease resistance. However, the sample requires complex pre-processing steps, including formamide, T4 DNA ligase, and DNA ligase and RNase inhibitor. In addition, the immobilized cells do not respond to the physiological activity of the cells in real time.

A widely used alternative method for telomerase activity detection is in situ fluorescence-based imaging, which tracks changes in the fluorescence signal of DNA extension sequences during telomerase-mediated elongation. However, naked DNA faces challenges in penetrating the cell membrane due to its anionic nature, as well as issues with lysosomal degradation and traversal of the nuclear pore [61]. To enhance the efficient intracellular delivery of DNA structures, liposome vectors were employed [62–64]. Wang et al. encapsulated DNA molecular beacons (MBs) into cationic liposomes with modification of nucleolin-specific aptamer AS1411 (Figure 1a). A single DNA MB can be rapidly self-assembled into a DNA dendrimer nanostructure by a single trigger step, which can be used for multistage fluorescence signal amplification and greatly simplifies the response process of the nanostructure [65]. Lipofectamine reagents are also widely used in delivering designed DNA fragments [66].

Most liposome/DNA delivery complexes are often trapped in endosomes and lysosomes after endocytosis, and many of these enzymes impair probe stability [67]. To address the initial problem of low uptake efficiency of DNA probes limiting detection sensitivity, various nanomaterial-based delivery systems have been developed. Other DNA functionalized nanomaterials are important tools for detection and controllable preparation, which have attracted more and more attention due to their potential applications in life sciences and nanomedicine. Qian et al. used mesoporous silicon dioxide nanoparticles (MSNs) as fluorescein carriers for fluorescent telomerase imaging [68]. The large pore volume and dissipation of MSNs enable them to load a large number of molecules. By trapping fluorescein in the mesopore of MSNs and covalently attaching the black hole fluorescence quenching agent (BHQ) to the inner wall of the mesopore, the detachment of DNA (O1) triggered fluorescein release, thereby 'turning on' its fluorescence (Figure 1b).

Gold nanoparticles (AuNPs) are considered ideal materials for intracellular target analysis and cell imaging due to their ability to immobilize and protect DNA, as well as their unique bio-compatibility [69]. In addition, as a quencher of fluorescent dyes via fluorescence resonance energy transfer (FRET), AuNPs enable the quantification and monitoring of telomerase activity in living cells using a one-step incubation technique (Figure 1c) [70]. To mitigate rapid photobleaching and autofluorescence of certain endogenous molecules, Zhao et al. employed bimetallic Au@Ag nanostructures as substrates that enhance surface-enhanced Raman scattering (SERS) while quenching fluorescence (Figure 1d). Telomerase initiated the extension of the primer chain, leading to the displacement of the Rhodamine 6G (R6G)-modified complementary strand through DNA strand displacement. This process restored fluorescence and diminished the SERS signal. Both the fluorescence intensity of R6G and the ratio of SERS-active molecule 4-p-mercaptobenzoic acid to R6G enabled in situ imaging and quantification of intracellular telomerase activity [71].

Additionally, the appropriate carrier can serve as a platform for precision therapy by leveraging the effects of metal ions on physiological activities. MnO₂ nanoplates have been used as nanocarriers as they not only protect DNA from degradation but also serve as a DNAzyme donor for Mn²⁺ for gene silencing (Figure 1e) [72]. Another example is platinum nanoparticles (PtNPs), which can be assembled with DNA icosahedra (PtNPs@DNA). These nanostructures entered the nucleus and induced DNA damage and cell death through the release of Pt ions. This platform holds great potential for targeted therapeutic applications in cancer, playing a dual role in both detection and treatment [73].

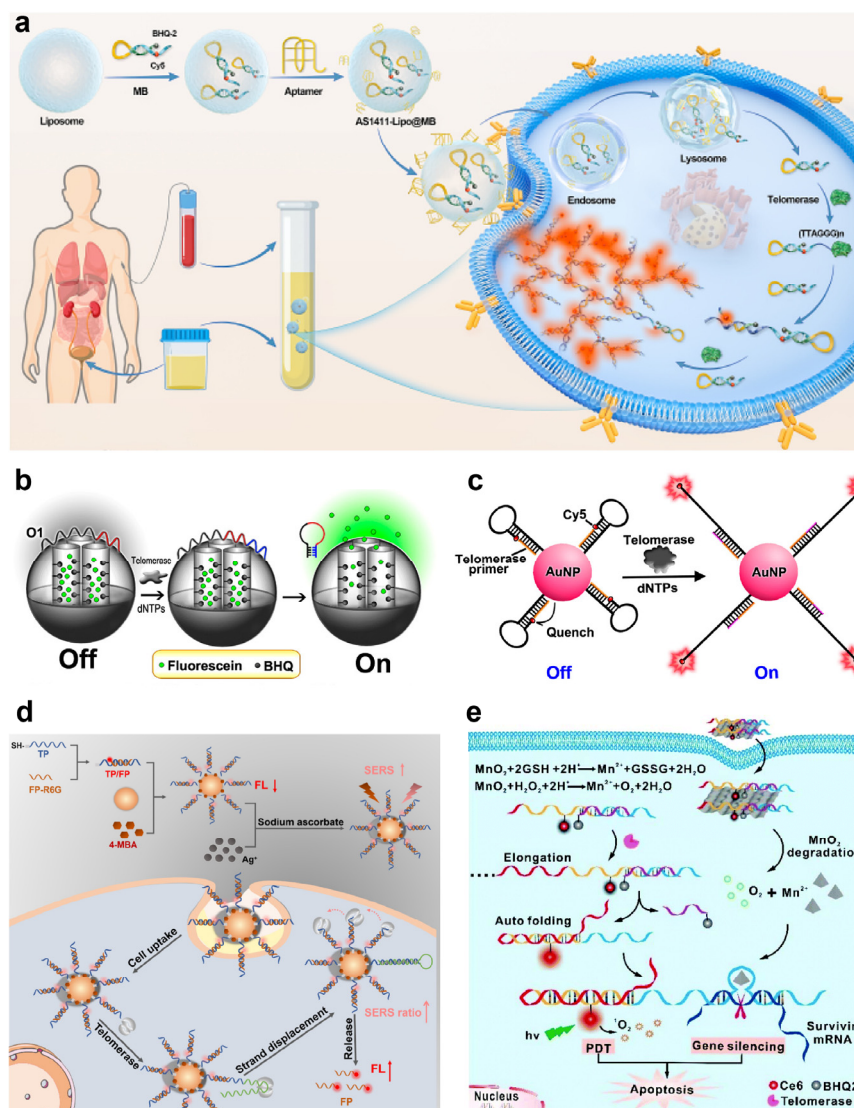


Figure 1. Schematic illustrations of advanced nanodevices for telomerase activity imaging detection. (a) Schematic of a single-molecule beacon-based arborescent nanoassembly for one-step in situ quantification of telomerase activity [65]. Copyright © 2024 American Chemical Society. (b) Schematic illustration of MSN probe-based intracellular analysis for telomerase activity detection [68]. Copyright © 2013 American Chemical Society. (c) Schematic illustration of nicked molecular beacon-functionalized gold nanoparticle probes for in situ analysis of intracellular telomerase activity [69]. Copyright © 2014 American Chemical Society. (d) Schematic of the target-activated fluorescence–SERS dual-signal imaging strategy for monitoring intracellular telomerase activity [70]. Copyright © 2023 Elsevier B.V. (e) Schematic of the synthesis of the DNA-MnO₂ theranostic nanodevice and its synergistic effect in cancer cells [72]. Copyright © 2020 Royal Society of Chemistry.

3. Luminescent Nanomaterials for Enhanced In Situ Telomerase Activity Detection

Other luminescent materials, such as quantum dots (QDs), metal nanomaterials, and various fluorescent materials, are being actively investigated for their enhanced luminescence properties. In particular, QDs are considered ideal alternative substitutes for organic fluorophores. QDs are semiconductor nanocrystalline materials with tunable emission, high quantum yield and extinction coefficient, long fluorescence lifetime, and photobleaching resistance [74,75]. These properties make QDs promising candidates for bioimaging and biosensing applications. For example, Ma et al. modified hairpin DNA with CdTe:Zn²⁺ QDs functionalized by a phosphorothioate, where QDs acted as a fluorophore [76]. Similarly,

Zhang et al. developed a novel self-assembled QD sensor directed by an entropy-driven reaction (Figure 2a). The assay system used two single-stranded DNAs (telomerase primer and fuel) and a triplex DNA (linker, blocker-1, blocker-2). In the presence of telomerase, the primer was extended, displacing blocker-1 and triggering DNA circuit amplification. This generated a Cy5-labeled DNA duplex that self-assembled on the 605QD surface, enabling FRET-based telomerase detection for real-time imaging in living cells without the need for separation or washing [77].

In an alternative approach, Jia et al. designed an amphiphilic nucleic acid probe with only fluorophore based on the aggregation-caused quenching (ACQ) effect (Figure 2b) [78]. When a hydrophilic DNA primer bound to the target, the dispersion of the hydrophobic fluorophore was enhanced, thereby increasing the fluorescent signal. In addition, the smaller molecular weight of the monomer compared to the DNA primer further aids in efficient signal amplification. Zhuang et al. combined positively charged aggregation-induced emission (AIE) dyes with substrate oligonucleotides to achieve in situ luminescence imaging and detection of telomerase activity in cells (Figure 2c) [79]. The positively charged AIE dye can bind to a negatively charged primer sequence. After the telomerase sequence was extended, the fluorescence signal could be turned on at the far end of the quenching agent. An AIEgens-modified probe (TPE-Py-DNA) served as a fluorescent reporter, and exonuclease III (Exo III) was used as a signal amplifier to detect telomerase activity (Figure 2d). After the extension of repeated sequences by telomerase, the released hydrophobic TPE-Py aggregated together and produced a related fluorescence signal, and the liberated product hybridized with another TPE-Py-DNA probe to start the second cycle [80]. Metal nanoclusters with AIE properties are also increasingly applied in telomerase activity detection. These nanoclusters offer excellent photostability in the intracellular environment, longer-wavelength emission, and efficient cell entry [81,82]. With sizes comparable to the electron Fermi wavelength, metal nanoclusters exhibit discrete electronic states and size-dependent fluorescence [83]. Due to their small size, facile synthesis, and excellent photostability, they have become widely utilized in fluorescence imaging applications [84]. Dong et al. developed an in situ biosynthetic method for oligonucleotide silver nanoclusters (AgNCs) (Figure 2e) [85]. AgNCs were synthesized using endogenous Glutathione (GSH); then, AgNO_3 was used as a precursor of the nanocluster, and the oligonucleotide of the cytosine (C) base served as a template. Because of the specific recognition ability between the G and C bases, the weak fluorescence of AgNCs became bright red emission when the nanocluster approached the guanine (G)-rich stem elongation product $(\text{TTAGGG})_n$. Similarly, Ran et al. exploited AIE to suppress the vibration and rotation of AuNC-DNA complexes, thus decreasing the rapid non-radiation deactivation and enhancing the ligand-to-metal charge transfer (Figure 2f). As a result, the fluorescence of the AuNCs could be enhanced, presenting AIE activity, making them promising candidates for telomerase activity detection [86].

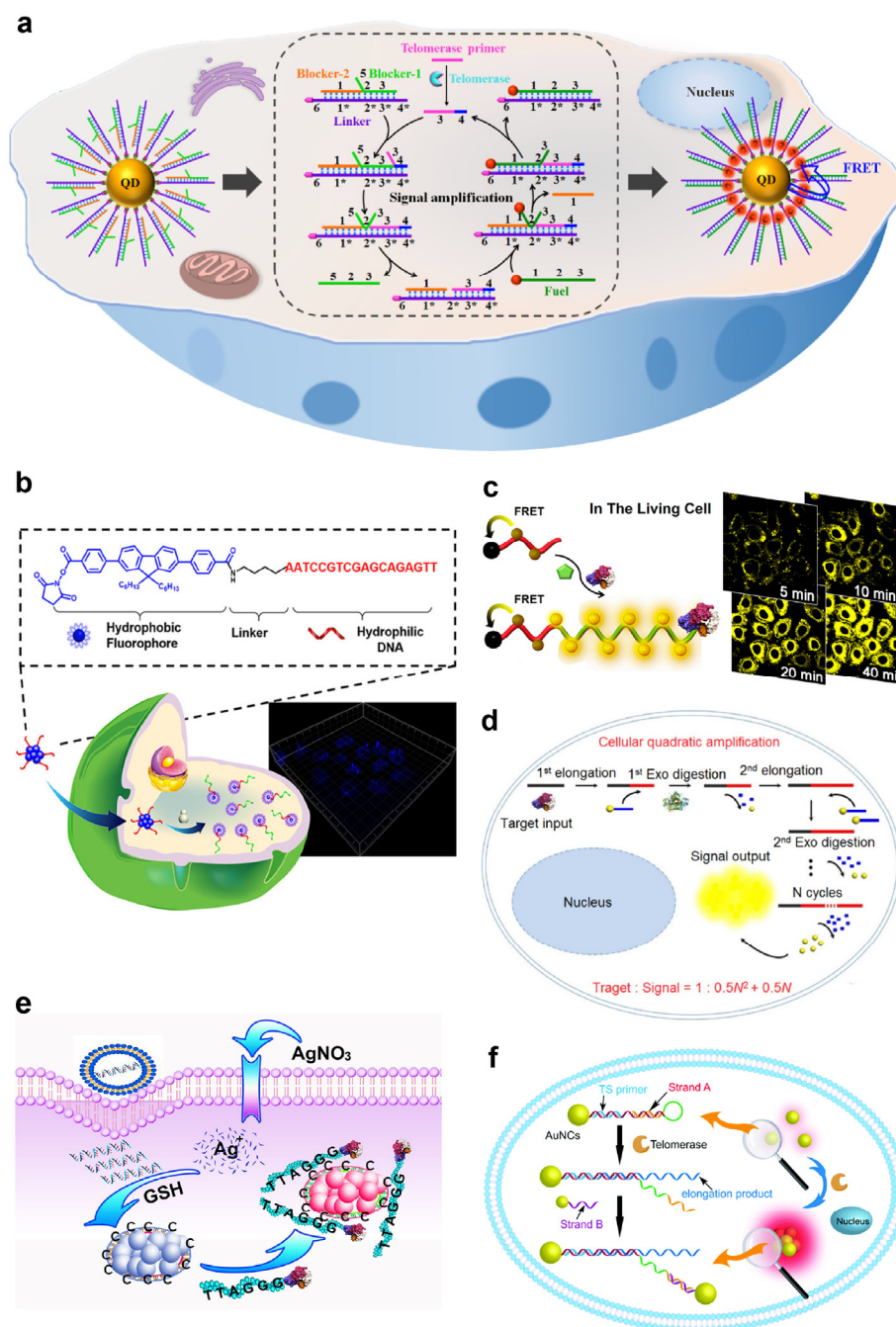


Figure 2. Advanced nanomaterial-based strategies for in situ telomerase activity detection and imaging. (a) Schematic of an entropy-driven self-assembly QD sensor for telomerase detection [77]. *: Complementary sequences to the blocker. Copyright © 2022 American Chemical Society. (b) Schematic of the ANAP for in situ detection of intracellular telomerase activity [78]. Copyright © 2016 American Chemical Society. (c) Schematic of AIE-based in situ detection and imaging of telomerase activity [79]. Copyright © 2016 American Chemical Society. (d) Schematic of a quadratic amplification strategy involving telomerase elongation and Exo III-assisted reaction [80]. Copyright © 2017 Elsevier B.V. (e) Schematic of AgNC biosynthesis for in situ imaging and biosensing of telomerase activity [85]. Copyright © 2017 American Chemical Society. (f) Illustration of telomerase-triggered DNA substitution and hybridization, leading to nanocluster assembly for visualizing telomerase activity [86].

4. Programmable DNA Systems for Cancer Diagnostics and Biosensing

4.1. DNA Logic Gates for Multi-Target Detection

Functioning as computational modules, logic gates process two or more inputs to perform specific operations and generate corresponding output signals. The concept of DNA logic gates gained significant attention in 1994, when DNA oligonucleotides were first used to perform logical operations and solve complex mathematical problems, sparking interest in developing biological logic gates [87]. DNA logic gates are capable of performing Boolean logic operations, which allow for the simultaneous detection of multiple tumor markers [88,89]. A signal is triggered only when all input conditions are met, offering a powerful tool for diagnostic applications.

Wang et al. employed DNA-based calculations of intracellular telomerase activity to construct a cascaded nucleic acid logic gate in response to intracellular telomerase (Figure 3A) [90]. The proposed strategy can be regarded as a cascaded logic circuit consisting of a YES gate and an AND gate. Telomerase substrate (TS) probes, which extend from intracellular telomerase, initiated the computational cascade as input in the first YES gate. The second input consisted of a partially complementary strand of the fluorophore-modified G_F strand, which separated the fluorophore and quencher-modified DNA duplex, generating a fluorescence signal when the conditions were met. In this work, a single input element of telomerase was used to construct AND gates, which can successfully distinguish tumor cells from normal cells.

Jiang et al. introduced a dual-input design for the simultaneous analysis of telomerase activity and miRNAs (Figure 3B) [91]. In the OR gate, incompletely complementary double strands were displaced either by telomerase-catalyzed elongation or miRNA-mediated strands, releasing single-stranded DNA to initiate the subsequent HCR reaction. In the AND gate, the telomerase extension sequence alone could not release single-stranded DNA from the three-way junction structure unless miRNA was also present. This dual-input design enabled the system to differentiate between various biomarkers in a single reaction. In addition to telomerase, Apurinic/Apyrimidinic endonuclease 1 (APE1, also called Ref-1) is an essential enzyme in the base excision repair pathway [92]. APE1 plays a role in telomere protection, and its deficiency is linked to telomere dysfunction and segregation defects in immortalized cells [93]. These cells maintain telomeres through mechanisms such as the alternative lengthening of telomeres (ALT) pathway [94,95]. It has been reported that both telomerase and APE1 are overexpressed in various cancer cells [96,97]. Fan et al. constructed a DNA fluorescence reporter based on a FRET DNA probe and nanoparticles with a cationic polymer shell to visualize the enzymatic activity of APE1 and telomerase (Figure 3C) [98]. This design enabled the two enzymes to be recognized and manipulated in an orthogonal manner without interference. In the presence of APE1, the AP site was rapidly cleaved, and the sequences elongated by telomerase complemented and formed a more stable DNA hairpin structure, inducing dissociation of the lysed strand, and realized AND-gate-controlled imaging of the two enzyme activities. Zhang et al. engineered this process on two gold nanoparticles of different sizes and designed a toehold-mediated strand displacement (TMSD) DNA nanomachine for simultaneous analysis of intracellular telomerase and APE1 (Figure 3D). In the presence of APE1, the intermediate ABW was unlocked and the telomerase toehold domain was exposed, which further triggered the accumulation of fluorescent signals [99].

In more complex applications, Zhu et al. constructed nanomachines with a continuous triple AND logic gate targeting the tumor microenvironment (TME), incorporating three distinct modules modified onto AuNPs, where fluorescence was initially quenched (Figure 3E). In the first logic process, tumor cells secrete matrix metalloproteinases (MMPs) during metastasis, cleaving the substrate peptides (P) and enabling DNA nanomachines

to enter the cells. Telomerase catalyzed primer extension, releasing a fuel strand and restoring the Cy3 fluorescence signal. This exposed a new toehold domain, which, upon interaction with miRNA-21, activated a secondary gate and released the Cy5 fluorescence signal. APE1 cleavage allowed the target (D) to be re-released, amplifying fluorescence and signal output [100]. This triple-input system enabled the detection of multiple cellular signals simultaneously, offering a sophisticated method for achieving understanding and diagnosis of tumor behavior in real time.

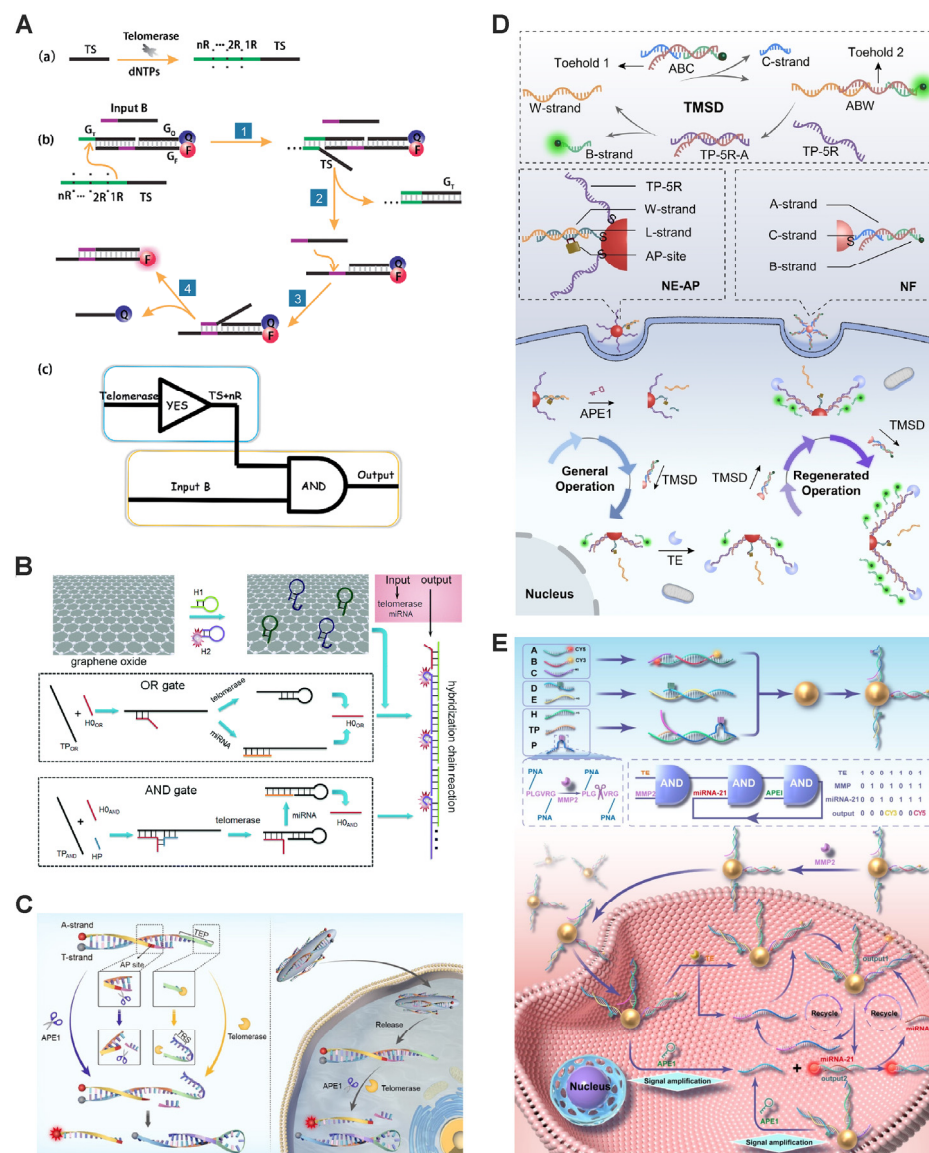


Figure 3. Advanced DNA nanomachines and logic gates for intracellular telomerase and enzyme activity imaging. (A) Schematic of DNA-based computation for imaging intracellular telomerase activity [90]. Copyright © 2016 Royal Society of Chemistry. (B) Schematic of AgNCs and HCR-based logic gates for detecting miRNA and telomerase activity [91]. Copyright © 2022 Royal Society of Chemistry. (C) Schematic of DNA-based fluorescent reporter for TE and APE1 activity imaging [98]. Copyright © 2021 Wiley-VCH GmbH. (D) Schematic of the TE-activated regenerative DNA nanomachine for intracellular APE1 sensing [99]. Copyright © 2022 Wiley-VCH GmbH. (E) Schematic of a triple-AND logic gate nanomachine for intracellular multi-enzyme tracking and miRNA detection [100]. Copyright © 2024 Elsevier B.V.

4.2. DNA Walkers in Multi-Target Biosensing

Due to their advantages of simple preparation, controllability, and predictability, DNA machines, such as DNA walkers, have been widely used in biosensors [101]. Programmable DNA structures can be designed into arbitrary shapes and controllable motions, which profit the construction of dynamic DNA nanodevices suitable for molecular-level movement [102]. The typical DNA walkers consist of three main elements: the driving forces, the walking strands, and the walking tracks [103]. The attachment of a target breaks the initial equilibriums of DNA walkers, driving the movement of the walking track and amplifying the signal output [104,105].

In biological processes like cell senescence and apoptosis, various proteins and DNA participate in the regulation and progression of these processes. Telomerase and flap endonuclease 1 (FEN1) are tumor marker enzymes closely related to the regulation of tumor progression and work in coordination [106–108]. FEN1 is involved in lagging strand DNA replication and base excision repair, playing a crucial role in maintaining genome stability in cancer cells. To monitor the activity of telomerase and FEN1 in living cells, Wang et al. proposed an in situ tracking-generated DNA walker for AND-gate logic imaging of these enzymes [109]. After the primer strands were elongated by telomerase, the walking strand hybridized to the tracks, invading the double-stranded track to form a triple-base overlapping structure, which could be specifically recognized by FEN1 (Figure 4a). Wang et al. designed a DNA walker on AuNPs using telomerase prolongation, which was triggered by APE1 [110]. APE1 played a crucial role as both a switch to initiate telomerase-mediated DNA elongation and a driver for the autonomous motion of the DNA nanomachine. APE1 cleaved the reporter probe, releasing a Cy5-labeled fragment from the AuNP. Simultaneously, the base-pairing instability between the remaining domain of the reporter probe caused the arm to swing, which then bound to a nearby reporter probe, initiating the next cleavage cycle (Figure 4b).

DNAzyme, first reported by Breaker and Joyce in 1994, is an artificially selected DNA sequence with catalytic functions [111]. With the addition of cofactors, DNAzymes will be primed and used for specific cleavage of DNA sequences [112]. Through the exponential enrichment (SELEX) technique, various DNAzymes have been developed as key components of biosensors, offering high affinity for specific targets and high selectivity against interference [113]. By combing DNAzyme with DNA walkers, Zhao et al. achieved high-sensitivity recognition of tumor cells versus normal cells [114]. This combination allowed the DNAzyme to confine reaction probes to nanoparticles, improving the efficiency of probe delivery into cells and stabilizing their local concentration [115]. The extension of the TP sequence caused by telomerase activated the swing arm and initiated the cleavage of the substrate in the presence of Mn^{2+} . The cleavage fragments then hybridized with another intact substrate on AuNPs, triggering another cycle of cleavage, release, and hybridization reactions (Figure 4c). Furthermore, p53 is a crucial tumor suppressor protein that regulates the cell cycle in human cells and is the most frequently mutated gene in cancer [116,117]. Mutations in p53 inactivate its tumor-suppressing function in about half of all cancers, promoting carcinogenesis and providing a growth advantage to cancer cells over normal cells [118,119]. Zhou et al. used the DNAzyme-powered DNA walker to visualize the cooperative expression of mutant p53 and telomerase in living cells [120]. The mutant p53 (Mup53)-activated unlocked substrates cleaved the substrate, triggering Cy5 fluorescence with Zn^{2+} . Telomerase primer (TSP) extension generated repeat sequences that opened the locking loop in the presence of Mg^{2+} , reactivating the DNAzyme. This substrate cleavage resulted in fluorescence recovery of FAM. The DNAzyme walking chain can continuously and autonomously cleave the corresponding substrate in the presence of metal ions, generating numerous fluorescent fragments (Figure 4d).

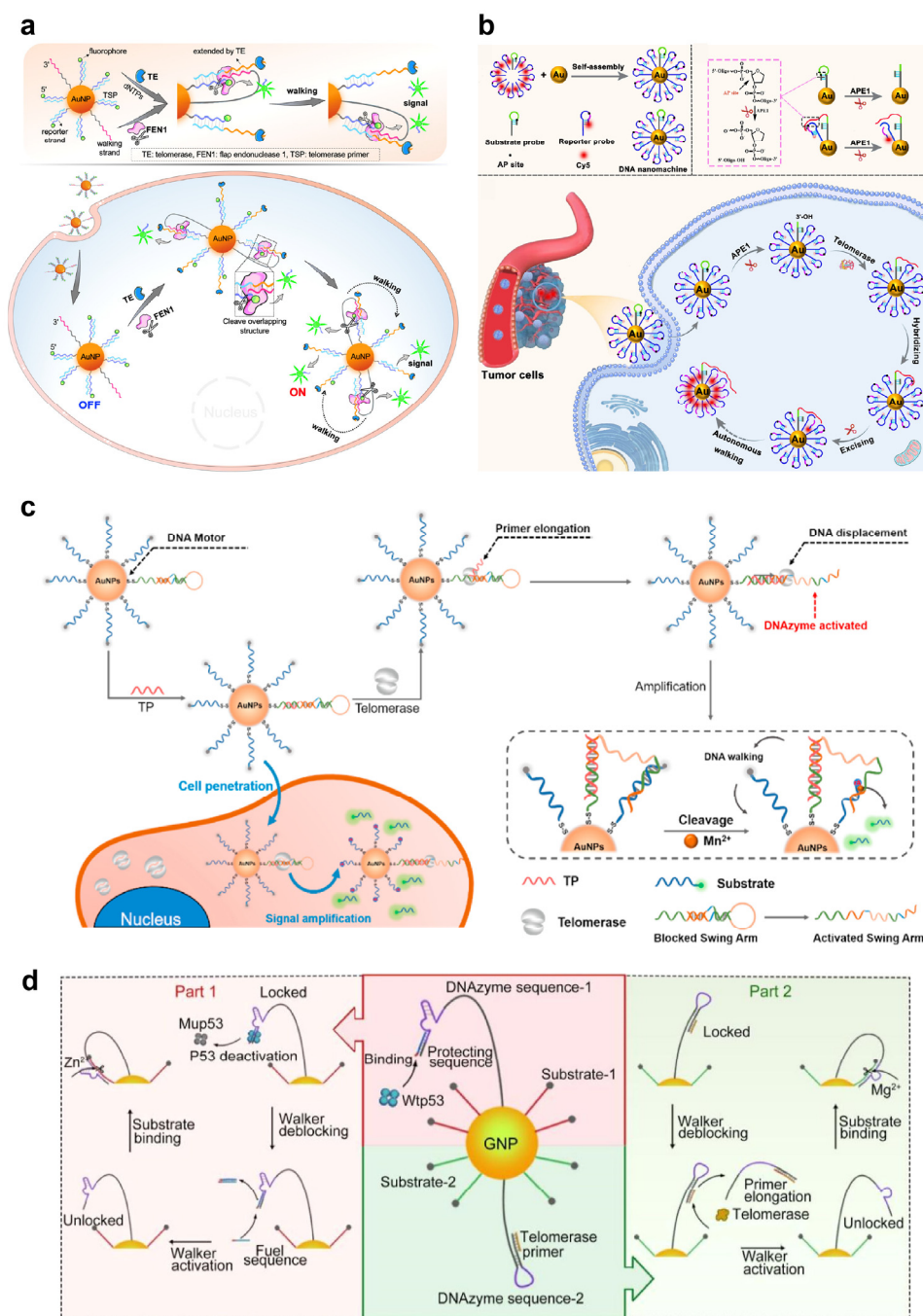


Figure 4. DNA nanomachines for real-time monitoring of telomerase and related enzyme activities in living cells. (a) Schematic of AND-gate DNA walker for imaging TE and FEN1 activities in single living cells [109]. Copyright © 2024 American Chemical Society. (b) Schematic of DNA nanomachine self-assembly, APE1 cleavage, and real-time telomerase imaging [110]. Copyright © 2024 American Chemical Society. (c) Schematic of TE-activated DNAzyme motor for monitoring telomerase activity in living cells [114]. Copyright © 2022 Elsevier B.V. (d) Schematic of DNAzyme-powered walker for mup53 and telomerase detection in living cancer cells [120]. Copyright © 2023 American Chemical Society.

5. Conclusions

Recent advancements in telomere and telomerase research have highlighted their crucial roles in cellular aging, cancer, and chromosomal stability. This review summarizes innovative biosensing techniques for monitoring and understanding telomerase activity

in living cells. These methods, including fluorescence imaging, electrochemical sensors, and DNA nanotechnology, enable in situ detection of telomerase activity, avoiding the need for cell extraction or lysis and addressing some of the limitations of conventional techniques. In addition, DNA-based biosensors have demonstrated high sensitivity and specificity in simultaneous monitoring of related cellular pathways. Through ingenious design, DNA probes have been endowed with the ability to store a lot of information and differentiate cells more precisely. Thus, these advanced biosensing strategies also support the development of precision therapies, providing the foundation for personalized cancer diagnostics and treatment. Future research will likely focus on the following areas:

1. Enhancing the real-time monitoring capabilities of biosensors: Integrating advanced nanomaterials with DNA-based probes holds significant potential to improve detection efficiency. Additionally, developing non-invasive imaging techniques for in vivo applications will be critical for clinical translation. However, there remains considerable potential for improving the biological safety and efficiency of delivery materials, which will aid in better understanding telomerase behavior throughout the cell cycle.
2. Developing innovative DNA probes: Due to the limited detection fragments of telomerase DNA, further development of probes targeting other sequences is needed. This can be achieved by analyzing and understanding telomerase-related DNA, allowing for more accurate detection of telomerase activity.
3. Expanding multiplexed biosensing systems: Although it is now possible to distinguish normal cells from cancer cells, accurately differentiating between various cancer cell types remains challenging. Detecting telomerase activity alongside other biomarkers could offer deeper insights into telomerase's role in aging and disease and provide new strategies for precise, cell-line-targeted therapies.

Author Contributions: Conceptualization, S.Z.; investigation, S.Z. and W.X.; writing—original draft preparation, S.Z. and W.X.; writing—review and editing, S.Z., S.X. and R.Q.; supervision, R.Q.; funding acquisition, R.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Science and Technology Commission of Shanghai Municipality (24DX1400200), the Shanghai Science and Technology Committee (22ZR1416800, 23ZR1416100), and the Fundamental Research Funds for the Central Universities to R.Q.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Blackburn, E.H. Structure and Function of Telomeres. *Nature* **1991**, *350*, 569–573. [[CrossRef](#)]
2. Aubert, G.; Lansdorp, P.M. Telomeres and Aging. *Physiol. Rev.* **2008**, *88*, 557–579. [[CrossRef](#)]
3. Greider, C.W.; Blackburn, E.H. Identification of a Specific Telomere Terminal Transferase Activity in Tetrahymena Extracts. *Cell* **1985**, *43*, 405–413. [[CrossRef](#)] [[PubMed](#)]
4. Dionne, I.; Wellinger, R.J. Cell Cycle-Regulated Generation of Single-Stranded G-Rich DNA in the Absence of Telomerase. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13902–13907. [[CrossRef](#)] [[PubMed](#)]
5. Monsen, R.C.; Chakravarthy, S.; Dean, W.L.; Chaires, J.B.; Trent, J.O. The Solution Structures of Higher-Order Human Telomere G-Quadruplex Multimers. *Nucleic Acids Res.* **2021**, *49*, 1749–1768. [[CrossRef](#)] [[PubMed](#)]
6. Cooke, H.J.; Brown, W.R.A.; Rappold, G.A. Hypervariable Telomeric Sequences from the Human Sex Chromosomes Are Pseudoautosomal. *Nature* **1985**, *317*, 687–692. [[CrossRef](#)] [[PubMed](#)]
7. Gardasevic, T.; Noy, A. The Impact of Sequence Periodicity on DNA Mechanics: Investigating the Origin of A-Tract's Curvature. *Nanoscale* **2024**, *16*, 18410–18420. [[CrossRef](#)]
8. Suvorova, Y.M.; Korotkova, M.A.; Korotkov, E.V. Comparative Analysis of Periodicity Search Methods in DNA Sequences. *Comput. Biol. Chem.* **2014**, *53*, 43–48. [[CrossRef](#)]
9. Tsonis, A.A.; Elsner, J.B.; Tsonis, P.A. Periodicity in DNA Coding Sequences: Implications in Gene Evolution. *J. Theor. Biol.* **1991**, *151*, 323–331. [[CrossRef](#)]

10. Shiekh, S.; Feldt, D.; Jack, A.; Kodikara, S.G.; Alfehaid, J.; Pasha, S.; Yildiz, A.; Balci, H. Protection of the Telomeric Junction by the Shelterin Complex. *J. Am. Chem. Soc.* **2024**, *146*, 25158–25165. [[CrossRef](#)]
11. Ghilain, C.; Gilson, E.; Giraud-Panis, M.J. Multifunctionality of the Telomere-Capping Shelterin Complex Explained by Variations in Its Protein Composition. *Cells* **2021**, *10*, 1753. [[CrossRef](#)] [[PubMed](#)]
12. Londoño Vallejo, J.A. Telomere Length Heterogeneity and Chromosome Instability. *Cancer Lett.* **2004**, *212*, 135–144. [[CrossRef](#)] [[PubMed](#)]
13. Jennings, B.J.; Ozanne, S.E.; Hales, C.N. Nutrition, Oxidative Damage, Telomere Shortening, and Cellular Senescence: Individual or Connected Agents of Aging? *Mol. Genet. Metab.* **2000**, *71*, 32–42. [[CrossRef](#)]
14. Whittemore, K.; Vera, E.; Martínez-Nevado, E.; Sanpera, C.; Blasco, M.A. Telomere Shortening Rate Predicts Species Life Span. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 15122–15127. [[CrossRef](#)] [[PubMed](#)]
15. Ruiz-Losada, M.; González, R.; Peropadre, A.; Gil-Gálvez, A.; Tena, J.J.; Baonza, A.; Estella, C. Coordination between Cell Proliferation and Apoptosis after DNA Damage in Drosophila. *Cell Death Differ.* **2022**, *29*, 832–845. [[CrossRef](#)] [[PubMed](#)]
16. Adhikary, U.; Paulo, J.A.; Godes, M.; Roychoudhury, S.; Prew, M.S.; Ben-Nun, Y.; Yu, E.W.; Budhraj, A.; Opferman, J.T.; Chowdhury, D.; et al. Targeting MCL-1 Triggers DNA Damage and an Anti-Proliferative Response Independent from Apoptosis Induction. *Cell Rep.* **2023**, *42*, 113176. [[CrossRef](#)] [[PubMed](#)]
17. Moix, S.; Sadler, M.C.; Kutalik, Z.; Auwerx, C. Breaking down Causes, Consequences, and Mediating Effects of Telomere Length Variation on Human Health. *Genome Biol.* **2024**, *25*, 125. [[CrossRef](#)]
18. Horikawa, I.; Cable, P.L.; Afshari, C.; Barrett, J.C. Cloning and Characterization of the Promoter Region of Human Telomerase Reverse Transcriptase Gene. *Cancer Res.* **1999**, *59*, 826–830.
19. Lingner, J.; Hughes, T.R.; Shevchenko, A.; Mann, M.; Lundblad, V.; Cech, T.R. Reverse Transcriptase Motifs in the Catalytic Subunit of Telomerase. *Science* **1997**, *276*, 561–567. [[CrossRef](#)] [[PubMed](#)]
20. Gazzaniga, F.S.; Blackburn, E.H. An Antiapoptotic Role for Telomerase RNA in Human Immune Cells Independent of Telomere Integrity or Telomerase Enzymatic Activity. *Blood* **2014**, *124*, 3675–3684. [[CrossRef](#)]
21. Kao, T.L.; Huang, Y.C.; Chen, Y.H.; Baumann, P.; Tseng, C.K. LARP3, LARP7, and MePCE Are Involved in the Early Stage of Human Telomerase RNA Biogenesis. *Nat. Commun.* **2024**, *15*, 5955. [[CrossRef](#)] [[PubMed](#)]
22. Kim, N.W.; Piatyszek, M.A.; Prowse, K.R.; Harley, C.B.; West, M.D.; Ho, P.L.C.; Coviello, G.M.; Wright, W.E.; Weinrich, S.L.; Shay, J.W. Specific Association of Human Telomerase Activity with Immortal Cells and Cancer. *Science* **1994**, *266*, 2011–2015. [[CrossRef](#)]
23. Han, X.; Zhang, Z.; He, L.; Zhu, H.; Li, Y.; Pu, W.; Han, M.; Zhao, H.; Liu, K.; Li, Y.; et al. A Suite of New Dre Recombinase Drivers Markedly Expands the Ability to Perform Intersectional Genetic Targeting. *Cell Stem Cell* **2021**, *28*, 1160–1176. [[CrossRef](#)]
24. Roake, C.M.; Chen, L.; Chakravarthy, A.L.; Ferrell, J.E.; Raffa, G.D.; Artandi, S.E. Disruption of Telomerase RNA Maturation Kinetics Precipitates Disease. *Mol. Cell* **2019**, *74*, 688–700. [[CrossRef](#)] [[PubMed](#)]
25. Theimer, C.A.; Jády, B.E.; Chim, N.; Richard, P.; Breece, K.E.; Kiss, T.; Feigon, J. Structural and Functional Characterization of Human Telomerase RNA Processing and Cajal Body Localization Signals. *Mol. Cell* **2007**, *27*, 869–881. [[CrossRef](#)]
26. Ghanim, G.E.; Sekne, Z.; Balch, S.; Van Roon, A.-M.M.; Nguyen, T.H.D. 2.7 Å Cryo-EM Structure of Human Telomerase H/ACA Ribonucleoprotein. *Nat. Commun.* **2024**, *15*, 746. [[CrossRef](#)] [[PubMed](#)]
27. Ghanim, G.E.; Fountain, A.J.; Van Roon, A.M.M.; Rangan, R.; Das, R.; Collins, K.; Nguyen, T.H.D. Structure of Human Telomerase Holoenzyme with Bound Telomeric DNA. *Nature* **2021**, *593*, 449–453. [[CrossRef](#)] [[PubMed](#)]
28. Zemora, G.; Handl, S.; Waldsich, C. Human Telomerase Reverse Transcriptase Binds to a Pre-Organized hTR in Vivo Exposing Its Template. *Nucleic Acids Res.* **2016**, *44*, 413–425. [[CrossRef](#)]
29. Martínez-Balsalobre, E.; García-Castillo, J.; García-Moreno, D.; Naranjo-Sánchez, E.; Fernández-Lajarín, M.; Blasco, M.A.; Alcaraz-Pérez, F.; Mulero, V.; Cayuela, M.L. Telomerase RNA-Based Aptamers Restore Defective Myelopoiesis in Congenital Neutropenic Syndromes. *Nat. Commun.* **2023**, *14*, 5912. [[CrossRef](#)]
30. Venteicher, A.S.; Abreu, E.B.; Meng, Z.; McCann, K.E.; Terns, R.M.; Veenstra, T.D.; Terns, M.P.; Artandi, S.E. A Human Telomerase Holoenzyme Protein Required for Cajal Body Localization and Telomere Synthesis. *Science* **2009**, *323*, 644–648. [[CrossRef](#)] [[PubMed](#)]
31. Choi, W.S.; Weng, P.J.; Yang, W. Flexibility of Telomerase in Binding the RNA Template and DNA Telomeric Repeat. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2116159118. [[CrossRef](#)] [[PubMed](#)]
32. Nakamura, T.M.; Cech, T.R. Reversing Time: Origin of Telomerase. *Cell* **1998**, *92*, 587–590. [[CrossRef](#)] [[PubMed](#)]
33. Muppirala, M.; Gupta, V.; Swarup, G. Emerging Role of Tyrosine Phosphatase, TCPTP, in the Organelles of the Early Secretory Pathway. *Biochim. Biophys. Acta Mol. Cell Res.* **2013**, *1833*, 1125–1132. [[CrossRef](#)]
34. Blasco, M.A.; Rizen, M.; Greider, C.W.; Hanahan, D. Differential Regulation of Telomerase Activity and Telomerase RNA during Multi-Stage Tumorigenesis. *Nat. Genet.* **1996**, *12*, 200–204. [[CrossRef](#)]
35. Liu, M.; Zhang, Y.; Jian, Y.; Gu, L.; Zhang, D.; Zhou, H.; Wang, Y.; Xu, Z. The Regulations of Telomerase Reverse Transcriptase (TERT) in Cancer. *Cell Death Dis.* **2024**, *15*, 90. [[CrossRef](#)] [[PubMed](#)]

36. Reilly, C.R.; Myllymäki, M.; Redd, R.; Padmanaban, S.; Karunakaran, D.; Tesmer, V.; Tsai, F.D.; Gibson, C.J.; Rana, H.Q.; Zhong, L.; et al. The Clinical and Functional Effects of TERT Variants in Myelodysplastic Syndrome. *Blood* **2021**, *138*, 898–911. [[CrossRef](#)] [[PubMed](#)]
37. Nandakumar, J.; Cech, T.R. Finding the End: Recruitment of Telomerase to Telomeres. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 69–82. [[CrossRef](#)]
38. Stoecklin, G.; Bukau, B. Telling Right from Wrong in Life—Cellular Quality Control. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 613–615. [[CrossRef](#)]
39. Wang, Y.; Gallagher-Jones, M.; Sušac, L.; Song, H.; Feigon, J. A Structurally Conserved Human and Tetrahymena Telomerase Catalytic Core. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 31078–31087. [[CrossRef](#)]
40. Wan, F.; Ding, Y.; Zhang, Y.; Wu, Z.; Li, S.; Yang, L.; Yan, X.; Lan, P.; Li, G.; Wu, J.; et al. Zipper Head Mechanism of Telomere Synthesis by Human Telomerase. *Cell Res.* **2021**, *31*, 1275–1290. [[CrossRef](#)]
41. Smith, E.M.; Pendlebury, D.F.; Nandakumar, J. Structural Biology of Telomeres and Telomerase. *Cell. Mol. Life Sci.* **2020**, *77*, 61–79. [[CrossRef](#)] [[PubMed](#)]
42. Autexier, C.; Lue, N.F. The Structure and Function of Telomerase Reverse Transcriptase. *Annu. Rev. Biochem.* **2006**, *75*, 493–517. [[CrossRef](#)] [[PubMed](#)]
43. Cheng, M.; Xiong, E.; Tian, T.; Zhu, D.; Ju, H.; Zhou, X. A CRISPR-Driven Colorimetric Code Platform for Highly Accurate Telomerase Activity Assay. *Biosens. Bioelectron.* **2021**, *172*, 112749. [[CrossRef](#)] [[PubMed](#)]
44. Wang, D.; Guo, R.; Wei, Y.; Zhang, Y.; Zhao, X.; Xu, Z. Sensitive Multicolor Visual Detection of Telomerase Activity Based on Catalytic Hairpin Assembly and Etching of Au Nanorods. *Biosens. Bioelectron.* **2018**, *122*, 247–253. [[CrossRef](#)]
45. Luo, S.; Zhang, Y.; Situ, B.; Zheng, L. Fluorescence Sensing Telomerase Activity: From Extracellular Detection to in Situ Imaging. *Sens. Actuators B* **2018**, *273*, 853–861. [[CrossRef](#)]
46. Ling, P.; Lei, J.; Ju, H. Nanoscaled Porphyrinic Metal–Organic Frameworks for Electrochemical Detection of Telomerase Activity via Telomerase Triggered Conformation Switch. *Anal. Chem.* **2016**, *88*, 10680–10686. [[CrossRef](#)] [[PubMed](#)]
47. Wang, Y.; Li, L.; Ge, S.; Zhang, L.; Wang, X.; Yu, J. DNzyme-Mediated Biodeposition Coupling Adjustable Cascade Electric Fields for Photoelectrochemical Telomerase Activity Monitoring. *ACS Sens.* **2023**, *8*, 3538–3546. [[CrossRef](#)]
48. Chen, S.; Zhao, J.; Shi, B.; Xu, C.; Zhao, S. An Ultrasensitive Chemiluminescence Strategy Based on a Microchip Platform for Telomerase Detection at a Single-Cell Level. *Chem. Commun.* **2021**, *57*, 3095–3098. [[CrossRef](#)]
49. Zhou, X.; Xu, M.; Huang, H.; Mazar, A.; Iqbal, Z.; Yuan, C.; Huang, M. An ELISA Method Detecting the Active Form of suPAR. *Talanta* **2016**, *160*, 205–210. [[CrossRef](#)]
50. Cengiz, M.; Sezer, C.V.; Gür, B.; Bayrakdar, A.; İzgördü, H.; Alanyalı, F.; Öziç, C.; Kutlu, H.M. The Role of Ceranib-2 and Its Nanoform on the Decrease of Telomerase Levels in Human Non-Small Cell Cancer. *Mol. Biol. Rep.* **2024**, *51*, 889. [[CrossRef](#)]
51. Li, C.; Chen, H.; Fan, T.; Zhao, J.; Ding, Z.; Lin, Z.; Sun, S.; Tan, C.; Liu, F.; Jiang, H.; et al. A Visualized Automatic Particle Counting Strategy for Single-cell Level Telomerase Activity Quantification. *VIEW* **2023**, *4*, e298. [[CrossRef](#)]
52. Duan, R.; Lou, X.; Xia, F. The Development of Nanostructure Assisted Isothermal Amplification in Biosensors. *Chem. Soc. Rev.* **2016**, *45*, 1738–1749. [[CrossRef](#)]
53. Wang, L.; Chen, C.; Huang, H.; Huang, D.; Luo, F.; Qiu, B.; Guo, L.; Lin, Z.; Yang, H. Sensitive Detection of Telomerase Activity in Cancer Cells Using Portable pH Meter as Readout. *Biosens. Bioelectron.* **2018**, *121*, 153–158. [[CrossRef](#)] [[PubMed](#)]
54. Latham, S.; Hughes, E.; Budgen, B.; Morley, A. Inhibition of the PCR by Genomic DNA. *PLoS ONE* **2023**, *18*, e0284538. [[CrossRef](#)] [[PubMed](#)]
55. Acharya, K.R.; Dhand, N.K.; Whittington, R.J.; Plain, K.M. PCR Inhibition of a Quantitative PCR for Detection of Mycobacterium Avium Subspecies Paratuberculosis DNA in Feces: Diagnostic Implications and Potential Solutions. *Front. Microbiol.* **2017**, *8*, 115. [[CrossRef](#)]
56. Yao, C.; Ou, J.; Tang, J.; Yang, D. DNA Supramolecular Assembly on Micro/Nanointerfaces for Bioanalysis. *Acc. Chem. Res.* **2022**, *55*, 2043–2054. [[CrossRef](#)]
57. Wang, W.; Lin, M.; Wang, W.; Shen, Z.; Wu, Z.S. DNA Tetrahedral Nanostructures for the Biomedical Application and Spatial Orientation of Biomolecules. *Bioact. Mater.* **2024**, *33*, 279–310. [[CrossRef](#)]
58. Cao, X.; Sun, Y.; Lu, P.; Zhao, M. Fluorescence Imaging of Intracellular Nucleases—A Review. *Anal. Chim. Acta* **2020**, *1137*, 225–237. [[CrossRef](#)] [[PubMed](#)]
59. Samanta, D.; Ebrahimi, S.B.; Mirkin, C.A. Nucleic-Acid Structures as Intracellular Probes for Live Cells. *Adv. Mater.* **2020**, *32*, 1901743. [[CrossRef](#)] [[PubMed](#)]
60. Li, X.; Liu, L.; Yin, F.; Liu, Y.; Xu, S.; Jiang, W.; Wang, R.; Xue, Q. In Situ Generation Spatial Confinement Fluorescence RNA for Sensitive and Stable Imaging of Telomerase RNA in Cells. *Sens. Actuators B* **2021**, *345*, 130414. [[CrossRef](#)]
61. Liang, H.; Chen, X.; Jin, R.; Ke, B.; Barz, M.; Ai, H.; Nie, Y. Integration of Indocyanine Green Analogs as Near-Infrared Fluorescent Carrier for Precise Imaging-Guided Gene Delivery. *Small* **2020**, *16*, 1906538. [[CrossRef](#)]

62. Zhang, J.; Li, Y.; Xiong, J.; Xu, H.; Xiang, G.; Fan, M.; Zhou, K.; Lin, Y.; Chen, X.; Xie, L.; et al. Delivery of pOXR1 through an Injectable Liposomal Nanoparticle Enhances Spinal Cord Injury Regeneration by Alleviating Oxidative Stress. *Bioact. Mater.* **2021**, *6*, 3177–3191. [[CrossRef](#)] [[PubMed](#)]
63. Wang, C.; Zhang, R.; He, J.; Yu, L.; Li, X.; Zhang, J.; Li, S.; Zhang, C.; Kagan, J.C.; Karp, J.M.; et al. Ultrasound-Responsive Low-Dose Doxorubicin Liposomes Trigger Mitochondrial DNA Release and Activate cGAS-STING-Mediated Antitumour Immunity. *Nat. Commun.* **2023**, *14*, 3877. [[CrossRef](#)]
64. Li, H.; Liu, Q.; Crielgaard, B.J.; De Vries, J.W.; Loznik, M.; Meng, Z.; Yang, X.; Göstl, R.; Herrmann, A. Fast, Efficient, and Targeted Liposome Delivery Mediated by DNA Hybridization. *Adv. Healthc. Mater.* **2019**, *8*, 1900389. [[CrossRef](#)] [[PubMed](#)]
65. Wang, Z.; Chen, X.; Qiu, X.; Chen, Y.; Wang, T.; Lv, L.; Guo, X.; Yang, F.; Tang, M.; Gu, W.; et al. High-Fidelity Sensitive Tracing Circulating Tumor Cell Telomerase Activity. *Anal. Chem.* **2024**, *96*, 5527–5536. [[CrossRef](#)]
66. Wang, Z.; Song, L.; Liu, Q.; Tian, R.; Shang, Y.; Liu, F.; Liu, S.; Zhao, S.; Han, Z.; Sun, J.; et al. A Tubular DNA Nanodevice as a siRNA/Chemo-Drug Co-delivery Vehicle for Combined Cancer Therapy. *Angew. Chem.* **2021**, *133*, 2626–2630. [[CrossRef](#)]
67. Degors, I.M.S.; Wang, C.; Rehman, Z.U.; Zuhorn, I.S. Carriers Break Barriers in Drug Delivery: Endocytosis and Endosomal Escape of Gene Delivery Vectors. *Acc. Chem. Res.* **2019**, *52*, 1750–1760. [[CrossRef](#)]
68. Qian, R.; Ding, L.; Ju, H. Switchable Fluorescent Imaging of Intracellular Telomerase Activity Using Telomerase-Responsive Mesoporous Silica Nanoparticle. *J. Am. Chem. Soc.* **2013**, *135*, 13282–13285. [[CrossRef](#)]
69. Li, L.; Xing, H.; Zhang, J.; Lu, Y. Functional DNA Molecules Enable Selective and Stimuli-Responsive Nanoparticles for Biomedical Applications. *Acc. Chem. Res.* **2019**, *52*, 2415–2426. [[CrossRef](#)] [[PubMed](#)]
70. Qian, R.; Ding, L.; Yan, L.; Lin, M.; Ju, H. A Robust Probe for Lighting Up Intracellular Telomerase via Primer Extension To Open a Nicked Molecular Beacon. *J. Am. Chem. Soc.* **2014**, *136*, 8205–8208. [[CrossRef](#)]
71. Zhao, Y.J.; Shen, P.F.; Fu, J.H.; Yang, F.R.; Chen, Z.P.; Yu, R.Q. A Target-Triggered Fluorescence-SERS Dual-Signal Nano-System for Real-Time Imaging of Intracellular Telomerase Activity. *Talanta* **2024**, *269*, 125469. [[CrossRef](#)] [[PubMed](#)]
72. Yi, J.T.; Pan, Q.S.; Liu, C.; Hu, Y.L.; Chen, T.T.; Chu, X. An Intelligent Nanodevice Based on the Synergistic Effect of Telomerase-Triggered Photodynamic Therapy and Gene-Silencing for Precise Cancer Cell Therapy. *Nanoscale* **2020**, *12*, 10380–10389. [[CrossRef](#)]
73. Ma, Y.; Wang, Z.; Ma, Y.; Han, Z.; Zhang, M.; Chen, H.; Gu, Y. A Telomerase-Responsive DNA Icosahedron for Precise Delivery of Platinum Nanodrugs to Cisplatin-Resistant Cancer. *Angew. Chem.* **2018**, *130*, 5487–5491. [[CrossRef](#)]
74. Park, S.; Song, S.; Kim, S.S.; Song, J. Charge Modulation Layer and Wide-Color Tunability in a QD-LED with Multiemission Layers. *Small* **2021**, *17*, 2007397. [[CrossRef](#)] [[PubMed](#)]
75. Lin, S.; Li, J.; Pu, C.; Lei, H.; Zhu, M.; Qin, H.; Peng, X. Surface and Intrinsic Contributions to Extinction Properties of ZnSe Quantum Dots. *Nano Res.* **2020**, *13*, 824–831. [[CrossRef](#)]
76. Ma, Y.; Mao, G.; Wu, G.; Fan, J.; He, Z.; Huang, W. A Novel Nano-Beacon Based on DNA Functionalized QDs for Intracellular Telomerase Activity Monitoring. *Sens. Actuators B* **2020**, *304*, 127385. [[CrossRef](#)]
77. Zhang, Q.; Yuan, Z.; Zhang, X.; Zhang, Y.; Zou, X.; Ma, F.; Zhang, C. Entropy-Driven Self-Assembly of Single Quantum Dot Sensor for Catalytic Imaging of Telomerase in Living Cells. *Anal. Chem.* **2022**, *94*, 18092–18098. [[CrossRef](#)]
78. Jia, Y.; Gao, P.; Zhuang, Y.; Miao, M.; Lou, X.; Xia, F. Facile Probe Design: Fluorescent Amphiphilic Nucleic Acid Probes without Quencher Providing Telomerase Activity Imaging Inside Living Cells. *Anal. Chem.* **2016**, *88*, 6621–6626. [[CrossRef](#)]
79. Zhuang, Y.; Huang, F.; Xu, Q.; Zhang, M.; Lou, X.; Xia, F. Facile, Fast-Responsive, and Photostable Imaging of Telomerase Activity in Living Cells with a Fluorescence Turn-On Manner. *Anal. Chem.* **2016**, *88*, 3289–3294. [[CrossRef](#)] [[PubMed](#)]
80. Min, X.; Xia, L.; Zhuang, Y.; Wang, X.; Du, J.; Zhang, X.; Lou, X.; Xia, F. An AIEgens and Exonuclease III Aided Quadratic Amplification Assay for Detecting and Cellular Imaging of Telomerase Activity. *Sci. Bull.* **2017**, *62*, 997–1003. [[CrossRef](#)] [[PubMed](#)]
81. Wu, Z.; Yao, Q.; Zang, S.; Xie, J. Aggregation-Induced Emission in Luminescent Metal Nanoclusters. *Natl. Sci. Rev.* **2021**, *8*, nwaa208. [[CrossRef](#)]
82. Zhou, M.; Du, X.; Wang, H.; Jin, R. The Critical Number of Gold Atoms for a Metallic State Nanocluster: Resolving a Decades-Long Question. *ACS Nano* **2021**, *15*, 13980–13992. [[CrossRef](#)] [[PubMed](#)]
83. Ding, J.; Yang, H.; Zhang, S.; Liu, Q.; Cao, H.; Luo, J.; Liu, X. Advances in the Electrocatalytic Hydrogen Evolution Reaction by Metal Nanoclusters-based Materials. *Small* **2022**, *18*, 2204524. [[CrossRef](#)] [[PubMed](#)]
84. Xiao, Y.; Wu, Z.; Yao, Q.; Xie, J. Luminescent Metal Nanoclusters: Biosensing Strategies and Bioimaging Applications. *Aggregate* **2021**, *2*, 114–132. [[CrossRef](#)]
85. Dong, F.; Feng, E.; Zheng, T.; Tian, Y. In Situ Synthesized Silver Nanoclusters for Tracking the Role of Telomerase Activity in the Differentiation of Mesenchymal Stem Cells to Neural Stem Cells. *ACS Appl. Mater. Interfaces* **2018**, *10*, 2051–2057. [[CrossRef](#)] [[PubMed](#)]
86. Ran, X.; Wang, Z.; Pu, F.; Ju, E.; Ren, J.; Qu, X. Nucleic Acid-Driven Aggregation-Induced Emission of Au Nanoclusters for Visualizing Telomerase Activity in Living Cells and In Vivo. *Mater. Horiz.* **2021**, *8*, 1769–1775. [[CrossRef](#)] [[PubMed](#)]
87. Adleman, L.M. Molecular Computation of Solutions to Combinatorial Problems. *Science* **1994**, *266*, 1021–1024. [[CrossRef](#)]

88. Zadegan, R.M.; Jepsen, M.D.E.; Hildebrandt, L.L.; Birkedal, V.; Kjems, J. Construction of a Fuzzy and Boolean Logic Gates Based on DNA. *Small* **2015**, *11*, 1811–1817. [[CrossRef](#)]
89. Peng, R.; Zheng, X.; Lyu, Y.; Xu, L.; Zhang, X.; Ke, G.; Liu, Q.; You, C.; Huan, S.; Tan, W. Engineering a 3D DNA-Logic Gate Nanomachine for Bispecific Recognition and Computing on Target Cell Surfaces. *J. Am. Chem. Soc.* **2018**, *140*, 9793–9796. [[CrossRef](#)] [[PubMed](#)]
90. Wang, W.; Huang, S.; Li, J.; Rui, K.; Bi, S.; Zhang, J.R.; Zhu, J.J. Evaluation of Intracellular Telomerase Activity through Cascade DNA Logic Gates. *Chem. Sci.* **2017**, *8*, 174–180. [[CrossRef](#)]
91. Jiang, Y.; Guo, Z.; Wang, M.; Cui, J.; Miao, P. Construction of Fluorescence Logic Gates Responding to Telomerase and miRNA Based on DNA-Templated Silver Nanoclusters and the Hybridization Chain Reaction. *Nanoscale* **2022**, *14*, 612–616. [[CrossRef](#)] [[PubMed](#)]
92. Wang, H.; Zhang, L.; Sun, H.; Xu, S.; Li, K.; Su, X. Screening and Application of Inhibitory Aptamers for DNA Repair Protein Apurinic/Apyrimidinic Endonuclease 1. *Int. J. Biol. Macromol.* **2023**, *242*, 124918. [[CrossRef](#)]
93. Kovalenko, O.A.; Caron, M.J.; Ulema, P.; Medrano, C.; Thomas, A.P.; Kimura, M.; Bonini, M.G.; Herbig, U.; Santos, J.H. A Mutant Telomerase Defective in Nuclear-cytoplasmic Shuttling Fails to Immortalize Cells and Is Associated with Mitochondrial Dysfunction. *Aging Cell* **2010**, *9*, 203–219. [[CrossRef](#)] [[PubMed](#)]
94. Madlener, S.; Ströbel, T.; Vose, S.; Saydam, O.; Price, B.D.; Demple, B.; Saydam, N. Essential Role for Mammalian Apurinic/Apyrimidinic (AP) Endonuclease Ape1/Ref-1 in Telomere Maintenance. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 17844–17849. [[CrossRef](#)]
95. Li, M.; Yang, X.; Lu, X.; Dai, N.; Zhang, S.; Cheng, Y.; Zhang, L.; Yang, Y.; Liu, Y.; Yang, Z.; et al. APE1 Deficiency Promotes Cellular Senescence and Premature Aging Features. *Nucleic Acids Res.* **2018**, *46*, 5664–5677. [[CrossRef](#)]
96. Zhang, L.; Luo, S.; Fan, R.; Li, R.; Li, W.; Chen, S.; Lan, F.; Zhu, Y.; Ji, T.; Zhang, Y.; et al. Localized Cas12a-Based Cascade Amplification for Sensitive and Robust Detection of APE1. *Talanta* **2024**, *280*, 126773. [[CrossRef](#)] [[PubMed](#)]
97. Tao, J.; Zhang, H.; Weinfeld, M.; Le, X.C. Development of a DNAzyme Walker for the Detection of APE1 in Living Cancer Cells. *Anal. Chem.* **2023**, *95*, 14990–14997. [[CrossRef](#)]
98. Fan, Z.; Zhao, J.; Chai, X.; Li, L. A Cooperatively Activatable, DNA-based Fluorescent Reporter for Imaging of Correlated Enzymatic Activities. *Angew. Chem. Int. Ed.* **2021**, *60*, 14887–14891. [[CrossRef](#)] [[PubMed](#)]
99. Zhang, Q.; Wang, Y.; Wang, W.; Min, Q.; Zhang, J.; Zhu, J. A Telomerase-Assisted Strategy for Regeneration of DNA Nanomachines in Living Cells. *Angew. Chem. Int. Ed.* **2023**, *62*, e202213884. [[CrossRef](#)]
100. Zhu, X.; Zhu, J.; Wang, J.; Wu, G.; Su, T.; Fan, L.; Li, J.; Liu, Y.; Yu, D.; Sun, R.; et al. A Reverberating Triple AND Logic Gate Nanomachine for Intracellular Multi-Enzyme Tracking and MicroRNA Detection. *Chem. Eng. J.* **2024**, *498*, 155631. [[CrossRef](#)]
101. Huang, J.; Zhu, L.; Ju, H.; Lei, J. Telomerase Triggered DNA Walker with a Superhairpin Structure for Human Telomerase Activity Sensing. *Anal. Chem.* **2019**, *91*, 6981–6985. [[CrossRef](#)] [[PubMed](#)]
102. Pulcu, G.S.; Mikhailova, E.; Choi, L.S.; Bayley, H. Continuous Observation of the Stochastic Motion of an Individual Small-Molecule Walker. *Nat. Nanotechnol.* **2015**, *10*, 76–83. [[CrossRef](#)] [[PubMed](#)]
103. Song, L.; Zhuge, Y.; Zuo, X.; Li, M.; Wang, F. DNA Walkers for Biosensing Development. *Adv. Sci.* **2022**, *9*, 2200327. [[CrossRef](#)] [[PubMed](#)]
104. Ji, Y.; Zhang, L.; Zhu, L.; Lei, J.; Wu, J.; Ju, H. Binding-Induced DNA Walker for Signal Amplification in Highly Selective Electrochemical Detection of Protein. *Biosens. Bioelectron.* **2017**, *96*, 201–205. [[CrossRef](#)] [[PubMed](#)]
105. Zhang, X.L.; Li, S.S.; Liu, Y.J.; Liu, W.W.; Kong, L.Q.; Chai, Y.Q.; Luo, X.L.; Yuan, R. High-Efficiency 3D DNA Walker Immobilized by a DNA Tetrahedral Nanostructure for Fast and Ultrasensitive Electrochemical Detection of MiRNA. *Anal. Chem.* **2023**, *95*, 4077–4085. [[CrossRef](#)] [[PubMed](#)]
106. Tang, Y.; Zhang, D.; Lu, Y.; Liu, S.; Zhang, J.; Pu, Y.; Wei, W. Fluorescence Imaging of FEN1 Activity in Living Cells Based on Controlled-Release of Fluorescence Probe from Mesoporous Silica Nanoparticles. *Biosens. Bioelectron.* **2022**, *214*, 114529. [[CrossRef](#)]
107. Zeng, X.; Qu, X.; Zhao, C.; Xu, L.; Hou, K.; Liu, Y.; Zhang, N.; Feng, J.; Shi, S.; Zhang, L.; et al. FEN1 Mediates miR-200a Methylation and Promotes Breast Cancer Cell Growth via MET and EGFR Signaling. *FASEB J.* **2019**, *33*, 10717–10730. [[CrossRef](#)] [[PubMed](#)]
108. Wang, C.; Zhang, D.; Tang, Y.; Wei, W.; Liu, Y.; Liu, S. Label-Free Imaging of Flap Endonuclease 1 in Living Cells by Assembling Original and Multifunctional Nanoprobe. *ACS Appl. Bio Mater.* **2020**, *3*, 4573–4580. [[CrossRef](#)] [[PubMed](#)]
109. Wang, C.; Zhang, Z.; Qiu, Y.; Bao, Y.; Song, Q.; Zou, B. In Situ Track-Generated DNA Walker for AND-Gate Logic Imaging of Telomerase and Flap Endonuclease 1 Activities in Living Cells. *Anal. Chem.* **2024**, *96*, 756–765. [[CrossRef](#)] [[PubMed](#)]
110. Wang, L.; Liu, W.; Wang, L.; Ho, Y.; Han, Y.; Li, D.; Zhang, C. Construction of an Enzymatically Controlled DNA Nanomachine for One-Step Imaging of Telomerase in Living Cells. *Anal. Chem.* **2024**, *96*, 4647–4656. [[CrossRef](#)]
111. Breaker, R.R.; Joyce, G.F. A DNA Enzyme That Cleaves RNA. *Chem. Biol.* **1994**, *1*, 223–229. [[CrossRef](#)] [[PubMed](#)]

112. Willner, I.; Shlyahovsky, B.; Zayats, M.; Willner, B. DNazymes for Sensing, Nanobiotechnology and Logic Gate Applications. *Chem. Soc. Rev.* **2008**, *37*, 1153–1165. [[CrossRef](#)]
113. McConnell, E.M.; Cozma, I.; Mou, Q.; Brennan, J.D.; Lu, Y.; Li, Y. Biosensing with DNazymes. *Chem. Soc. Rev.* **2021**, *50*, 8954–8994. [[CrossRef](#)] [[PubMed](#)]
114. Zhao, Y.J.; Liao, Y.; Fu, J.H.; Li, Y.Z.; Zhu, Y.L.; Chen, Z.P.; Yu, R.Q. Telomerase-Initiated Three-Dimensional DNzyme Motor for Monitoring of Telomerase Activity in Living Cells. *Biosens. Bioelectron.* **2023**, *219*, 114757. [[CrossRef](#)]
115. Zhou, Q.; Li, T.; Li, X.; Wei, L.; Luo, J.; Bai, L.; Duan, W.J.; Xie, B.; Sun, B.; Chen, J.X.; et al. Dual miRNA-Triggered DNA Walker Assisted by APE1 for Specific Recognition of Tumor Cells. *Anal. Chem.* **2024**, *96*, 6774–6783. [[CrossRef](#)] [[PubMed](#)]
116. Chang, F.; Syrjänen, S.; Syrjänen, K. Implications of the P53 Tumor-Suppressor Gene in Clinical Oncology. *J. Clin. Oncol.* **1995**, *13*, 1009–1022. [[CrossRef](#)]
117. Nigro, J.M.; Baker, S.J.; Preisinger, A.C.; Jessup, J.M.; Hosteller, R.; Cleary, K.; Signer, S.H.; Davidson, N.; Baylin, S.; Devilee, P.; et al. Mutations in the P53 Gene Occur in Diverse Human Tumour Types. *Nature* **1989**, *342*, 705–708. [[CrossRef](#)]
118. Zhou, S.; Ran, J.; Man, S.; Zhang, J.; Yuan, R.; Yang, X. Exploring the Effect of Steric Hindrance on Trans-Cleavage Activity of CRISPR-Cas12a for Ultrasensitive SERS Detection of P53 DNA. *Anal. Chem.* **2024**, *96*, 10654–10661. [[CrossRef](#)] [[PubMed](#)]
119. Xhafa, S.; Di Nicola, C.; Tombesi, A.; Pettinari, R.; Pettinari, C.; Scarpelli, F.; Crispini, A.; La Deda, M.; Candreva, A.; Garufi, A.; et al. Pyrazolone-Based Zn(II) Complexes Display Antitumor Effects in Mutant P53-Carrying Cancer Cells. *J. Med. Chem.* **2024**, *67*, 15676–15690. [[CrossRef](#)] [[PubMed](#)]
120. Zhou, Z.R.; Li, D.W.; Qian, R.C.; Ju, H. DNzyme-Powered DNA Walker for Cooperative Expression Imaging of Mutant P53 and Telomerase in Cancer Cells. *Anal. Chem.* **2023**, *95*, 4122–4130. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.