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Recent progress in electrochemiluminescence biosensors based on DNA nanostructures



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Highlights:

- DNA nanostructures boost ECL biosensing via precise interfacial engineering and signal amplification.
- Signal amplification strategies of DNA nanostructures in ECL biosensing.
- Applications span nucleic acids, proteins, small molecules, and intracellular biomarkers.

Abstract: Electrochemiluminescence (ECL) combines electrochemical triggering with optical readout, giving it high sensitivity, low background, and good spatiotemporal control. DNA nanostructures are attractive in this context because their sequences, shapes, and functional groups can be programmed with nanometer precision. This review surveys how DNA nanotechnology has been used to improve ECL biosensors. We first outline annihilation and co-reactant ECL mechanisms, and then use a structure-activity framework to compare 1D, 2D, and 3D DNA architectures in terms of interfacial ordering, donor-acceptor distance control, and nanoconfinement. We next examine amplification strategies, including HCR, CHA, RCA, and DNA walkers, and discuss DNAzymes, logic circuits, and CRISPR-Cas cascades as emerging functional modules. Applications are grouped by analyte class, covering pathogen nucleic acids, microRNAs, circulating tumor DNA, protein biomarkers, small molecules, metal ions, and intracellular markers. Finally, we highlight challenges related to stability in biological fluids, scalable fabrication, *in vivo* compatibility, and the sensitivity-dynamic range trade-off, and suggest future directions such as machine-learning-guided DNA nanoengineering, adaptive single-cell ECL platforms, and point-of-care integration.

Keywords: ECL; DNA nanostructures; ECL biosensing

1. Introduction

Biosensing is important in disease detection, biological research and environmental examination [1,2]. Compared with other detecting methods, ECL is unique because it uses electrochemical control to generate an optical signal, which provides high sensitivity, low background and precise temporal-spatial



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resolution [3,4]. This property enables the determination of nucleic acids, proteins and other biomacromolecules, and has been recently applied to the analysis of single cells and *in situ* imaging. However, it is difficult to apply ECL from preliminary experimental tests to reliable analytical devices. The same confined reactions and the dependence on the interface and signal transduction which affect the sensitivity of ECL also hinder the molecular arrangement at the interface and signal transmission. Therefore, the ordinary ECL systems still have some difficulties such as ineffective electron transfer, random probe orientation, restricted single mode amplification and lack of structural flexibility for multiplexed or logic-gated detection [5–7].

DNA nanostructures have received considerable attention due to their combining characteristics of atom-by-atom programming and good biocompatibility which allow specific functionalization. These features are suitable for structural frameworks and also as active elements in biological detection [8–12]. In ECL biosensing, DNA nanostructures can perform three main roles: modifying the interface, efficient signal amplification and intelligent functional combination. The amplification has been enhanced by employing dynamic devices like DNA walkers and catalytic circuits [13], and by including molecular recognition specificity and signal processing ability via DNA logical circuits [14] and CRISPR-Cas cascade systems [15]. Therefore, the research field is developing from basic “signal on/off” tests to more complicated and comprehensive biochemical analytical systems.

Based on the present situation, this review describes a comprehensive and critical study about the recent progress in DNA nanostructure-based ECL biosensing (Figure 1). Firstly, the basic ECL reaction mechanisms, such as annihilation and co-reactant pathways, are introduced to provide the physical and chemical foundation for the following discussion. Secondly, a unified model of structure-activity relationship is developed to explain how one-dimensional (1D), two-dimensional (2D) and three-dimensional (3D) DNA nanostructures affect the ECL performance by means of precise interface engineering, distance control and confinement effects. According to these structural features, the existing signal amplification techniques including hybridization chain reaction (HCR), catalytic hairpin assembly (CHA), rolling circle amplification (RCA) and DNA walking systems are evaluated, and the new functional units like DNAzymes, logical circuits and CRISPR-Cas integrated cascades are also considered, emphasizing their operating principles, performance comparisons and cooperation with DNA templates. From the basic mechanisms to practical applications, the common biosensor platforms are classified according to their detection objects: nucleic acids, proteins, small molecules and intracellular markers, and during this classification, the reasonable design of structures is shown to enhance the diagnostic efficiency. At last, an objective evaluation of the major problems in this field is given, such as structural stability, large-scale production, *in vivo* compatibility and clinical use, and future research directions including the application of machine learning (ML) in DNA nanoengineering and the establishment of adaptive ECL systems for single-cell analysis and dynamic biomarker monitoring are proposed.

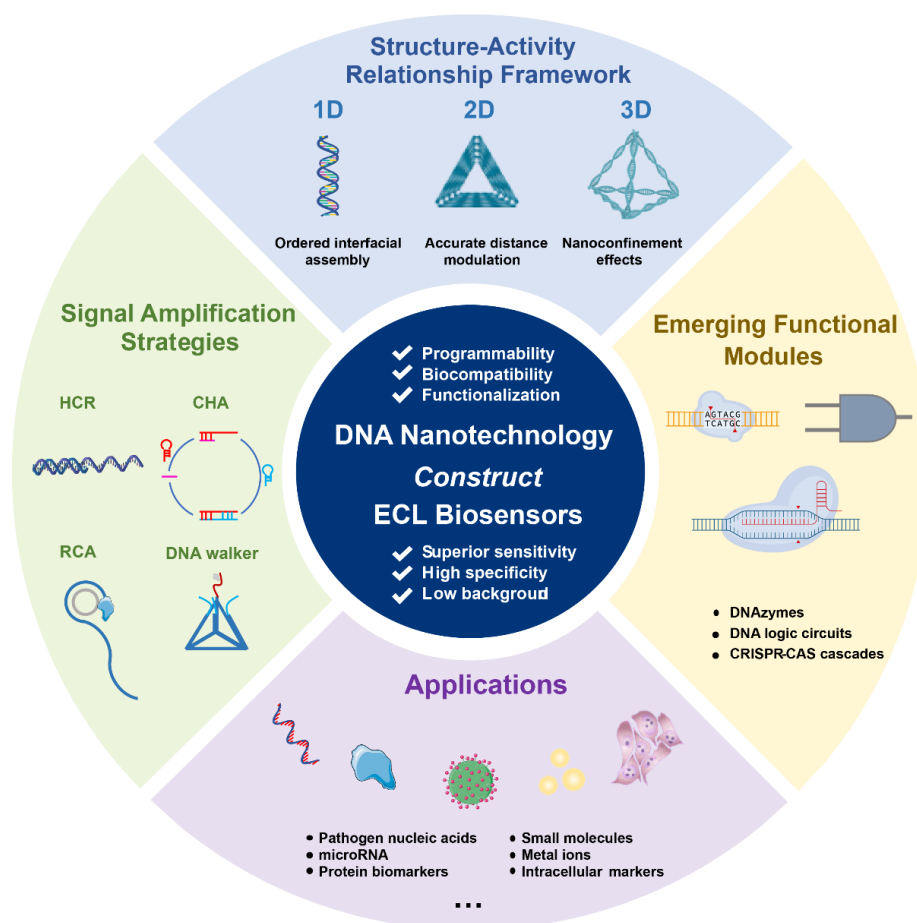


Figure 1. Schematic of ECL biosensors based on DNA nanostructure.

2. Fundamental principles and mechanisms of ECL

2.1. Principles and characteristics of ECL

ECL is a light emission resulted from electrochemical reactions, where the exergonic electron transfer at the electrode surface results in the release of light for the excited-state substances [16]. By integrating electrochemistry and chemiluminescence, ECL has high sensitivity, specific detection, fast response, a broad linear range and low background interference. The electrode potential can be accurately adjusted to detect trace substances precisely, and selecting proper reaction systems with electrode materials can improve the selectivity and reduce the interferences [17,18]. Its rapid reaction kinetics is suitable for real-time monitoring applications [19], and the good relationship between ECL intensity and the analyte concentration allows for quantitative detection over a wide range of concentrations [20]. In addition, the potential-controlled reaction mechanism can effectively reduce the background noise, thereby enhancing the signal-to-noise ratio and increasing the detection sensitivity [21].

2.2. Reaction mechanism of ECL

ECL is produced at the electrode surface from the radical species and the reaction processes can be divided into the annihilation mechanism and the co-reactant mechanism depending on the method of radical generation. In the former mechanism, the radicals originate from only one fluorescent molecule,

whereas in the latter mechanism, the reactions occur between the fluorescent molecules and the co-reactant molecules through bimolecular electrochemical reactions [22].

2.2.1. Annihilation mechanism

The annihilation mechanism includes electron transfer between the electro-generated anions and cations at the electrode interface, resulting in the formation of excited states and eventually causing light emission. The whole reaction pathway is mathematically explained in Equations (1)–(7) [23]. If the enthalpy of the annihilation is greater than the energy required for the formation of singlet excited state ($1R^*$), it will form directly. For example, the radical annihilation process of 9,10-diphenylanthracene (DPA) molecules occurs in ECL systems [24]. When the enthalpy change of the annihilation reaction is less than the energy needed to generate the lowest singlet excitation from the ground state but more than the triplet energy level, a triplet excited state ($3R^*$) is firstly produced and then converted into $1R^*$ by triplet annihilation.

Singlet state excitation process:

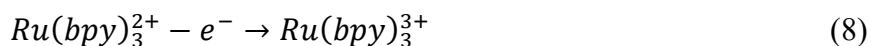


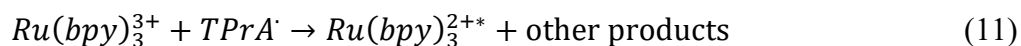
Triplet state excitation process:



2.2.2. Co-reactant mechanism

By contrast, co-reactant mechanisms rely on solution-phase reagents during unidirectional potential scanning. Depending on the polarity of the applied potential, luminophores and co-reactants undergo sequential oxidation or reduction to generate radical intermediates. The decomposition products of co-reactants act as strong reductants or oxidants, which then react with electrogenerated luminophore species to form excited states. Based on the redox characteristics of the intermediate species, co-reactant ECL systems fall into oxidative-reduction and reductive-oxidation types. The classic $Ru(bpy)_3^{2+}/TPrA$ system developed by Bard's group serves as a representative example of this mechanism [25–27], with key reaction steps as follows:





Although the co-reactant ECL systems have high sensitivity, their efficiency in complex matrices is usually decreased because of the reduction in signal and low quantum yield. These disadvantages cannot be eliminated merely by modifying the luminous substance or the co-reactant; it is essential to specially design the electrode interface and the local reaction conditions. DNA nanostructures are suitable for this purpose as the surface coating, the location of the probe and the spatial distribution can be controlled with a precision of several nanometres. Therefore, they offer a controllable way to improve both the interfacial structure and the reaction rates in ECL systems.

3. DNA nanostructures for controlling ECL signals

3.1. Characteristics of DNA nanostructures

DNA nanostructures belong to a newly developed type of nanomaterials with special properties such as structural programmability and molecular precision, which have gained much attention in various fields. Especially in biomedical applications, considerable progress has been made in biosensing and therapeutic delivery systems due to the gradual realization of their practical value [28–32].

3.1.1. Structural programmability

The double-helix structure of DNA, assembled through complementary base pairing (A-T and G-C) across its four nucleotide building blocks adenine, thymine, guanine and cytosine, endows DNA nanostructures with excellent structural programmability. Recent breakthroughs in programmable assembly have enabled precise modulation of DNA strand interactions, allowing researchers to construct nanostructures with tailored geometries and dimensions. Computer-aided design tools such as caDNAno and CanDo have revolutionized structural prediction and optimization workflows in this field [33]. For example, 1D DNA nanowires are fabricated via rational sequence design of single-stranded DNA (ssDNA). Through precise multi-strand DNA assembly, 2D DNA nanosheets including the widely used DNA origami structure can be formed, and this design strategy can be further extended to build complex 3D DNA nanostructures such as DNA tetrahedra and octahedra.

3.1.2. Biocompatibility and functionalization

As internal biological molecules, the DNA nanostructures possess good biocompatibility and low immunogenicity and are appropriate for being incorporated into various biological systems in medicine. At present, the major research focuses on studying their distribution *in vivo* for a long time and conducting thorough safety tests. The improvement of functionalization techniques has extensively extended the applications of DNA nanostructures. By using phosphoramidite chemistry and click reactions, the precise combination of groups like fluorophores, biotin and thiol groups can now be achieved with a precision of less than one nanometer, thus allowing for high-density functional modifications of the DNA

framework. The incorporation of fluorescent groups makes the DNA nanostructures suitable as bio-imaging agents, which helps to observe their distribution and function in living organisms [34]. The biotin modification enables the specific recognition and capture of certain biomolecules through the efficient biotin-avidin binding interaction [35]. The thiol groups allow the covalent attachment of gold nanoparticles to the DNA backbone, forming new hybrid nanostructures with adjustable plasmonic properties [36]. Up to now, biosensors based on modified DNA nanostructures have been successfully applied to detect various biomarkers at very low concentrations, indicating their practical values [37].

3.2. DNA nanostructures in different dimensions

Dimensionality is more than a geometric descriptor for DNA nanostructures; it directly shapes ECL performance by influencing signal amplification efficiency, spatial resolution, and interfacial stability [12,38]. In the discussion that follows, 1D, 2D, and 3D architectures are therefore examined in turn, with particular attention to how their structural features give rise to distinct functional roles in ECL biosensing. To clarify how dimensionality itself governs ECL behavior, Section 3.2.4 provides a direct comparison among 1D, 2D, and 3D DNA nanostructures, and Table 1 summarizes this comparison in terms of five key parameters: probe density, steric hindrance, electron transfer, structural stability, and signal amplification efficiency.

Table 1. Comparison of 1D, 2D, and 3D DNA nanostructures in terms of the five key parameters affecting ECL performance.

Parameter	1D DNA nanostructures	2D DNA nanostructures	3D DNA nanostructures
Probe density	Low to moderate; linear arrangement; limited surface area	High; dense planar packing; large surface area-to-volume ratio	Moderate to high effective density; ordered 3D arrangement
Steric hindrance	Low along linear axis, but limited capture area	High at high probe density; crowding reduces target accessibility	Low; solution-like accessibility; reduced crowding
Electron transfer	Moderate; depends on scaffold conductivity and probe orientation	Moderate; planar crowding can hinder electron transfer	Enhanced; ordered orientation
Structural stability	Moderate; susceptible to nuclease degradation unless modified	Moderate to high in buffer; nuclease sensitivity remains	High; tetrahedra resist nuclease/thermal denaturation; hydrogels tunable
Signal amplification efficiency	Moderate; compatible with HCR/RCA	Strong but crowding-limited; compatible with HCR/CHA	Strongest; nanoconfinement accelerates kinetics

3.2.1. 1D DNA nanostructures

1D DNA nanostructures mainly take the form of nanowires and nanotubes. Early work in this area centered on developing fabrication methodologies for these architectures, and steady technological advances have delivered finer control over DNA strand assembly, leading to improved structural stability and homogeneity. In 2004, Liu's group pioneered the self-assembly of DNA nanotubes from three-way junction tiles, which acted as templates for conductive nanowires and demonstrated the potential of DNA self-assembly for building programmable 1D structures [39]. Later, Park's group realized length-controlled silver nanowires through triplex-helix DNA tile assembly, marking a key milestone in programmable

1D nanostructure construction [40]. More recently, the integration of RCA and HCR techniques has driven advances in the functionalization and scalable production of 1D DNA nanostructures [41], broadening their applicability in ECL biosensing.

Early ECL biosensing platforms relied heavily on hairpin-based recognition. Zhang and co-workers reported the first selective ECL biosensor for ssDNA, in which a hairpin DNA served as the recognition element and a ruthenium complex acted as the signal generator [42]. In the absence of target, the surface-confined probe remained folded, keeping its termini close to the electrode and producing a strong ECL signal. Hybridization with the target ssDNA converted the stem-loop into a linear duplex, moving the label away from the electrode and thereby decreasing the ECL response. Subsequent work shifted toward linear ECL resonance energy transfer (ECL-RET) probes. Ru's group, for instance, designed an acetamidine (ACE) biosensor that combined ECL-RET with DNA recycling amplification and used Zr-based metal-organic frameworks (Zr-MOFs) as emitters [43]. In this system, target-induced aptamer dissociation initiated DNA cascade reactions that first enhanced ECL-RET and then led to signal quenching, giving a detection limit of 6.0 pg/mL. DNA nanowires offer another route: their conductivity and molecular recognition capability allow target hybridization to modulate interfacial electron transfer and thus ECL signal transduction. Wang's group exploited silver nanowires to build a dual-signal amplified ECL biosensor for Pb^{2+} , achieving a detection limit of 0.33 pM [44]. Mohammad's group combined RCA with DNA nanowire assemblies to construct ratiometric ECL/electrochemical sensors for miRNA-18a, with linearity from 20 aM to 50 pM and a sensitivity of 9 aM [45].

In general, 1D DNA nanostructures offer an easy and adaptable basis for ECL biosensing, possessing advantages such as simple preparation, definite linear structure and suitability for different amplification methods. Nevertheless, their small surface area and restricted spatial arrangement do not meet the requirements for complicated multiplexed assays and intracellular uses, which leads to further study on higher dimensional structures.

3.2.2. 2D DNA nanostructures

2D DNA nanostructures differ from their 1D counterparts in that they extend across a planar interface and offer a higher surface area-to-volume ratio. These characteristics make them especially attractive for biosensing tasks that require dense probe immobilization or simultaneous recognition of multiple targets. The field was fundamentally changed by Rothemund's 2006 demonstration of DNA origami, in which a long scaffold strand is folded into prescribed 2D patterns through hybridization with hundreds of short staple strands [46]. Later advances in tile-based assembly [47] and fractal assembly [48] further increased the structural complexity of 2D DNA nanostructures and broadened the range of functionalization strategies available to them.

In the ECL biosensing systems, 2D DNA nanostructures can serve as both immobilization supports and signal amplifiers at the same time. For example, Y-shaped DNA frameworks have been used to improve the signal strength [49], and DNA machines based on CHA can detect the target with high sensitivity [50]. Because of their large surface area, the nano sheets can immobilize DNA probes at a high density, thereby significantly increasing the capturing ability of the targets [51,52].

However, 2D DNA nanostructures have inherent limitations for further practical development; therefore, 3D DNA nanostructures have been proposed to address these drawbacks. When the probes are placed closely on a flat surface, obvious spatial congestion is found and the adjacent recognition

units are disturbed each other stably [53]. Such congestion results in the decrease of target accessibility and reduces the hybridization rate, which leads to an increase in non-specific adsorption from complicated biological media. In practical detection, these problems usually occur together: the blocked target diffusion, the decreased probe availability and the background adsorption all affect the sensitivity and repeatability. Thus, there exists a contradiction in the 2D DNA nanostructures. They can offer a high probe density and strong signal output, but their flat structure always influences the molecular accessibility and flexibility under complex test conditions. This contradiction indicates the advantage of building higher dimensional structures.

3.2.3. 3D DNA nanostructures

According to the previous discussion, 2D structures have some disadvantages in terms of molecular accessibility and spatial adaptability. 3D DNA nanostructures provide an alternative method to overcome these problems by possessing higher structural complexity and volumetric spatial arrangement [54]. As an important area of research in DNA nanotechnology, 3D architectures exhibit increased structural stiffness, strengthened multi-valency binding ability and the capability to contain or arrange functionally loaded substances in limited areas, thus being more suitable for ECL biosensing in complicated biological media and *in vivo* environments.

In the 3D DNA structures, the DNA tetrahedron has been frequently studied and applied. It forms a stable pyramid framework through the base pairing of four single DNA strands, and its resistance to nuclease digestion and heat denaturation, together with the four special geometric vertices which are advantageous for orthogonal functionalization, makes it an ideal support for various biosensing and targeted delivery systems [55]. Some more complex three dimensional designs like octahedra [56] and dodecahedra [57] have biomimetic spatial organization characteristics and might lead to the development of new ECL biosensors. Nevertheless, the stability of these structures is not absolute and is greatly reduced in complicated biological environments. Experimental data indicate that the half-life of DNA tetrahedra in 20% human serum is approximately 23.9 hours, and in 50% diluted human serum is around 10.1 hours [58]. In fetal bovine serum, a 20 bp DNA tetrahedron degrades about four times faster than a 13 bp one, possibly due to the exposure of more double stranded regions which are easily recognized by nucleases [59]. Beyond nuclease-mediated breakdown, DNA nanostructures are also destabilized by the low divalent cation levels typical of physiological fluids. Most DNA nanostructures need 5–20 mM Mg^{2+} to maintain their structural integrity, but common physiological media like RPMI-1640 contain only around 0.4 mM of combined Mg^{2+} and Ca^{2+} ; this cation shortage triggers electrostatic repulsion between strands and leads to partial disassembly [60], and nonspecific adsorption of serum proteins can further perturb structure and reduce stability. To address these issues, a range of stabilization strategies have been developed. For instance, chemical modification of DNA strands [61] or oligolysine-based surface coatings can greatly improve DNA's resistance to degradation [60], and metallointercalators that insert into DNA's major or minor grooves can also markedly boost the serum stability of DNA tetrahedra [62]. These approaches together form a practical toolkit for improving DNA nanostructure stability in ECL biosensing applications, though the trade-off between enhanced stability and retained sensing performance needs to be carefully weighed for each use case.

In ECL biosensing, 3D DNA nanostructures make use of their 3D architecture to accomplish two major functions. One of them is to act as rigid spatial supports which increase the accessibility of

molecules. Compared to the closely packed flat surfaces in 2D assemblies, the tetrahedral frameworks project the recognition probes outwards from the electrode surface and into the bulk solution, thus reducing steric hindrance and improving the accessibility of the targets. The clear 3D arrangement also helps to decrease the non-specific adsorption and enhances the collision rate between probes and target molecules. For example, Yu and his colleagues designed a CRISPR/Cas12a based ECL biosensor combined with DNA tetrahedra for detecting HPV-16 [63]. In comparison with the traditional linear or planar arrangements of probes, the modified interface with tetrahedra reduces the steric hindrance and improves the cleavage efficiency of Cas12a, allowing sensitive detection without prior amplification of the target nucleic acid.

Another important function of these 3D structures is to function as responsive materials in smart interfacial engineering. Cross-linked DNA hydrogels, another type of 3D DNA structures, have tunable porosity and volume changes under different stimuli, which makes them appropriate as gate layers to control mass transport and catalytic accessibility at electrode interfaces. Ma and his team developed a target responsive DNA hydrogel for this purpose, using it as an interfacial gate layer to regulate mass transport and catalytic access [64]. This 3D DNA network effectively inhibits the non-specific background leakage while providing many recognition sites, indicating the great potential of DNA hydrogels in smart interfacial engineering. Besides hydrogels, cross-linked 3D DNA networks can also be used as molds for *in situ* synthesis of ECL active nanomaterials. Jin's group fabricated one 3D DNA network by hybridizing PCR products with gold nanoparticle labeled probes, serving as a scaffold for the controllable deposition of silver nanoclusters [65].

In conclusion, 3D DNA nanostructures have certain advantages over their lower dimensional counterparts: rigid tetrahedral supports improve the target accessibility and enzyme activity, 3D nano machines accelerate the reaction kinetics through spatial confinement, and stimuli responsive hydrogels enable the active regulation of interfacial mass transport. However, these advantages come together with greater synthesis complexity, higher material costs and difficulties in batch-to-batch reproducibility. Moreover, the large hydrodynamic volume of some 3D structures might slow down the diffusion and mass transport in solution phase sensing. Nevertheless, the strong structural and functional abilities of 3D DNA nanostructures ensure their position as the most promising platform for high performance ECL biosensing. In the following section, we will study how the structural features discussed in this chapter can be combined with nucleic acid amplification techniques to further enhance the sensitivity of detection.

3.2.4. Comparative analysis of dimensionality effects on ECL performance

Due to the various types of 1D, 2D and 3D DNA nanostructures discussed in the previous sections, a comparative study is advantageous in order to understand the effect of dimensionality on the whole ECL performance. The changes in performance are due to several related factors such as the probe loading density and spatial distribution, steric hindrance and target accessibility, the efficiency of interfacial electron transfer, the structural stability and the signal amplification ability. The dimensionality does not exclusively decide these factors, but it influences the basic geometric and mechanical structure where the probe spacing, orientation, local concentration and molecular transport can be reasonably arranged.

The 1D DNA nanostructures, such as nanowires, nanotubes and other linear arrangements, cause the functional units to be mainly along a single spatial axis. This simple topological property enables the directional arrangement and adjustment of distances without raising the overall construction complexity.

The probe loading capacity and target accessibility are less affected by the dimensionality itself than by the length, surface configuration and the functionalization density of the basic framework. Likewise, the efficiency of interfacial electron transfer is dependent on the position and orientation of the redox- or ECL-active substances relative to the electrode surface. Since they are easy to fabricate and suitable for amplification methods like HCR and RCA, the 1D structures are especially suitable for applications requiring simple preparation and directional signal transmission.

2D DNA nanostructures, like DNA origami and planar DNA assemblies, provide enough space for different recognition units and signal components to be arranged with definite lateral distances. The main advantage is not only the dense arrangement of probes, but also the ability to change the number of probes according to the needed space. If the distance between probes is determined incorrectly, it might cause steric hindrance and decrease the accessibility of the target. However, by means of rational design of 2D structures, these problems can be resolved by controlling the positions at the nanometre level. The wide space also allows for multiple recognitions and placement of several amplification parts. Nevertheless, their 2D form may have some disadvantages such as surface immobilisation, mass transport and structural heterogeneity, so 2D structures are particularly suitable for tasks needing spatially programmed, high-capacity or multiplexed molecular organization.

3D DNA nanostructures such as DNA tetrahedra, polyhedra, networks and hydrogels organize the functional components in 3D space, providing an additional control over the probe orientation, accessibility and the local molecular environment. For electrode-bound DNA tetrahedra, the stable framework pushes the recognition probes away from the electrode surface with a definite orientation and spacing, which decreases the steric hindrance at the surface and improves the accessibility of the targets. Such a spatial arrangement can also improve the electron transfer at the interface when it optimizes the distance and orientation between the redox- or ECL-active substances and the electrode. The compact structure like DNA tetrahedra usually possess higher structural rigidity and biostability compared to the flexible single-stranded probes, while DNA hydrogels and interconnected networks offer adjustable porosity and 3D loading capacity. Moreover, 3D confinement can increase the effective local concentration of the reaction components and reduce the diffusion distances, thus accelerating the reaction kinetics and leading to significant signal amplification when combined with appropriate DNA reaction systems.

A summary of these dimensionality-dependent effects is presented in Table 1. This comparison shows that dimensionality is more than a structural descriptor, as it acts as a key governing factor for the balance between probe density, accessibility, electron transfer, stability and amplification gain. In practical applications, the optimal dimensionality should be selected according to the analytical target, sample matrix, required sensitivity and acceptable operational complexity.

3.3. Regulatory roles of DNA nanostructures in ECL systems

DNA nanostructures can eliminate the drawbacks of the present ECL systems by utilizing some special regulatory mechanisms, making full use of their ability to be arranged spatially and modified at particular sites. At the molecular level, these structures can achieve accurate control over the luminescence efficiency, reaction kinetics and the stability of the entire system, which is advantageous for constructing sensitive ECL biosensors [66,67]. The regulatory roles of DNA nanostructures in ECL systems mainly include three aspects.

First, DNA nanostructures can control the resonance energy transfer distance in ECL by using the Förster resonance energy transfer (FRET) principle. The energy transfer efficiency is affected by the distance between the donor and acceptor pairs. Compared to the random arrangement of electrodes, the designed DNA network places the lumiphores and acceptor groups at specific distances, providing a feasible and reversible method to modify the ECL intensity. The distance-dependent regulation mechanism acts as a reliable molecular gauge to build the ECL biosensor interface. Yang's group has developed an *in situ* quenching-type ECL biosensor for detecting DNA methylation using bimetallic RuCu-MOFs as signal sources and an interconnected DNA walker as signal converters [68]. After bisulfite treatment, the methylated target DNA functions as the moving strand to separate the immobilized hairpin fuel strands (H1, H2, H3-Fc) on the electrode surface and form a 3D DNA layer, resulting in many ferrocene labels near the RuCu-MOFs. The interaction between the RuCu-MOFs and ferrocene leads to efficient ECL quenching, enabling the detection of methylated DNA with a sensitivity approximately one order of magnitude and a detection limit of 14 aM. In this system, the DNA walker performs the task of changing the distance; each movement alters the distance between donor and acceptor by a certain amount, thus controlling the energy transfer step by step. 1D DNA nanowires and hairpins restrict the displacement in a straight line, while 2D DNA origami ensures the accurate arrangement of various components in a plane. 3D DNA polyhedra offer control in the third dimension, providing more opportunities for positioning the donors and acceptors.

Second, the arrangement of DNA nanostructures in a certain order improves the electron transfer rate. The probes attached randomly on the electrode surface usually have an unfavorable orientation and steric hindrance, resulting in inefficient electron transfer. The rigid DNA nanostructures such as tetrahedrons act as spatial supports to fix the bio-recognition probes on the electrode surface with a controlled orientation and packing density. This ordered arrangement can decrease the steric hindrance, reduce the nonspecific adsorption of biological molecules from complex matrices and accelerate the electron transfer between the electrode and the luminescent luminophores, thus enhancing both the reaction efficiency and the signal reproducibility. For instance, Tian's group has designed an improved electron transfer ECL biosensor for the detection of methylated Septin9 gene in colorectal cancer [69]. By immobilizing DNA tetrahedrons on the substrate, the biosensor can minimize the nonspecific adsorption and preserve the ordered arrangement of probes, which is beneficial in capturing the methylated DNA targets effectively and significantly strengthening the ECL signal. The 3D DNA tetrahedrons project the recognition probes outward into the bulk solution with well-defined, nanometer scale distances between the probes (approximately 5–10 nm), which reduces the steric obstruction and increases the accessibility of the target. Some typical studies indicate that this spatial configuration can increase the binding rate of the target by about one order of magnitude [70].

Third, the nanoconfinement effect in DNA nanostructures can increase the local reactant concentration and improve the catalytic activity. By restricting the reactants in a small volume, DNA nanostructures reduce the diffusion distances, enhance the collision frequency and speed up the molecular reactions, thereby accelerating the overall reaction kinetics. For instance, interconnected DNA nanostructures can gather many CRISPR activator sequences in a limited area which increases the binding efficiency between Cas12a and its activators by 1.5 times compared with the dispersed linear sequences [71]. Additionally, circular DNA molecules without free ends have better exonuclease resistance which strengthens the structural stability of DNA-based detection systems in complex

biological media. In a DNA tetrahedron-mediated catalytic hairpin assembly (DTCHA) system, the effective local concentration of hairpin probes is about 147 μM , which is about 1000 times higher than the bulk concentration. The nanoconfinement effect reduces the reaction time by 6.4 times and increases the initial reaction rate by 12.4 times in comparison with the standard CHA [72]. Furthermore, the geometric shape of DNA nanostructures has a great influence on the degree of nanoconfinement. 1D structures place the reactants along a straight framework providing only slight spatial restrictions. 2D flat structures include several reaction components within a certain area which increases their effective local concentration and decreases the lateral diffusion distances. However, 3D structures such as DNA polyhedra, nanoworlds and gels confine the reactants in a 3D nanoscale space, leading to higher effective local concentrations and shorter diffusion paths, and thus promoting more efficient reaction kinetics.

Although the three regulatory mechanisms are described individually above, they usually work together in one DNA nanostructure. For example, a DNA tetrahedron can regulate simultaneously the distance between donor and acceptor, the orientation of the probe and the local reactant concentration. It is not easy to separate these individual effects completely, but their relative importance can be studied qualitatively by means of control experiments. The influence of the distance regulation can be separated by changing the donor-acceptor separation with rigid DNA spacers without altering the probe density and the local conditions; under these circumstances, the distance-dependent energy transfer efficiency can be examined based on the Förster relationship. The influence of the ordered interface assembly can be determined by comparing the randomly arranged and specifically arranged probes with the same surface density and donor-acceptor distance. The effect of the confinement can be evaluated by comparing confined and unconfined systems under the same distance, orientation and bulk concentration, using kinetic parameters like the initial reaction rate and half-life to indicate a confinement factor. Consequently, these three mechanisms should be considered as coupled factors affecting the total performance instead of independent additive effects.

In summary, the three regulatory mechanisms function at distinct but complementary levels. Distance modulation directly controls the energy transfer efficiency between ECL donors and acceptors; interfacial ordering ensures recognition elements are presented on the electrode surface in an optimal configuration; and nanoconfinement accelerates the chemical steps of the ECL reaction itself. The full realization of these regulatory effects, however, depends critically on the dimensionality and structural integrity of the DNA scaffolds employed. In the following section, we systematically examine how DNA nanostructures of different dimensions ranging from 1D nanowires to 3D tetrahedra and hydrogels translate these mechanistic principles into practical ECL biosensing performance.

4. Signal amplification strategies of DNA nanostructures in ECL biosensing

Owing to their programmable base-pairing interactions and versatile assembling properties, DNA nanostructures are not only applied as passive structural frameworks but also act as active devices for signal amplification. When combined with nucleic acid amplification reactions, they can generate a stronger ECL signal which is better than that produced by DNA nanostructures alone. In this section, both the conventional amplification techniques such as HCR, CHA, RCA and DNA walker systems and the novel cascade platforms including several amplification units are introduced. The working principles of these methods are explained, and their effects on DNA nanostructures under different experimental conditions are also compared. A table showing some typical applications is given in Table 2.

Table 2. Analytical application of signal amplification strategies.

Signal Amplification Strategies	Amplification Time	Reaction Steps	Background Leakage	Enzyme Requirement	Analytes	Linear Range	LOD	Real Sample Recovery	Reference
HCR	0.5–2 h	1 step	low to moderate	No	hepatitis B virus DNA	10 fM–10 nM	7.41 fM	97.4%–108%	[73]
					miRNA-499	1 fM–1 nM	0.42 fM		[74]
					miRNA-221	100 aM–100 pM	5.25 aM		[75]
					ctDNA	10 aM–10 nM	2.74 aM	97.45%–104.15%	[76]
					miRNA-21	10 aM–1 pM	10 aM		[77]
					miRNA-155	10 aM–1 pM	10 aM		
					ts3011a RNA	10 fM–100 nM	3.33 fM	97.2%–103%	[78]
CHA	1–2 h	1 step	moderate	No	miRNA-21	1 aM–1 nM	0.02 aM	96%–102.45%	[79]
					target DNA	10 fM–10 nM	1.09 fM	92.02%–101.62%	[80]
					miRNA-21	1 aM–1 nM	0.6 aM	95%–102.4%	[81]
					miRNA-21	10 fM–100 nM	6.2 fM	98%–101.2%	[82]
						1 fM–10 nM	0.48 fM		
RCA	1–3 h	2 steps	low	Yes	HIV DNA	100 aM–100 pM	27.0 aM	96.32%–110.4%	[83]
					apurinic/aprimidinic endonuclease 1	1×10^{-8} – 1×10^{-2} U/ μ L	2.29×10^{-9} U/ μ L	95.5%–107.2%	[84]
					has-miR-10a-5p	1 fM–1 nM	1.23 fM	92%–107%	[85]
					PSA	10 fg/mL–100 ng/mL	2.3 fg/mL	94.6%–101.1%	[86]
					miRNA Let-7a	10 fM–10 nM	2.7 fM		
DNA Walker	0.5–2 h	Multiple step	low to moderate	Yes or No	target DNA	100 aM–1 nM	25.1 aM	93%–105.3%	[87]
					miRNA Let-7a	10 fM–100 nM	4.92 fM		[88]
					exosomes	240–74,000 particles/ μ L	185 particles/ μ L	96.4%–106.2%	[89]
					amyloid- β oligomers	1 pM–10 nM	0.62 pM	93%–104%	[90]
					soluble urokinase-type plasminogen activator receptor	0.7 pg/mL–80.0 ng/mL	0.344 pg/mL		[91]
					miRNA-21	50 aM–100 pM	7.55 aM		[92]
					acetamiprid	10 fM–1 μ M	4.58 fM		[93]
DNAzyme	0.5–2 h	1–2 steps	low to moderate	No	c-myc mRNA	10 aM–10 nM	74.3 aM	95.3%–103.3%	[94]
					Cu ²⁺	0.1 nM–10 mM	0.025 nM	95.07%–106.6%	[95]
					miRNA-222	100 aM–100 pM	12.90 aM		[96]
					17 β -estradiol	0.01 ng/mL–200 ng/mL	3.27 fg/mL	97%–105.9%	[97]
CRISPR-Cas cascade	1–3 h	2–3 steps	low	Yes	α -synuclein oligomer	0.01 fM–1 pM	8.3 aM	97%–104.3%	[98]
					citrus Huanglongbing pathogen	10 fM–1 nM	2 fM	97.2%–110%	[99]
					N-nitrosodimethylamine	10 pg/mL–10 μ g/mL	5.33 pg/mL	93.84%–97.48%	[100]
					miR-543	10 fM–10 nM	6.91 fM	94.9%–106%	[101]

Note: LOD values are derived from calibration curves.

4.1. Enzyme-free nucleic acid amplification

4.1.1. Hybridization chain reaction

HCR is an isothermal amplification technique that requires no enzymes and relies on DNA strand displacement. A typical HCR system includes two or more metastable hairpin DNA probes. When an initiator strand is present, usually a DNA sequence related to the target, it opens the stem loop structure of hairpin H1. The single stranded region that becomes exposed then hybridizes with the complementary domain of hairpin H2, which opens H2 in turn. The opened H2 can then hybridize with another H1 molecule. This chain reaction proceeds on its own, creating a long double stranded DNA polymer and giving a linear amplification profile [102–104]. In ECL biosensing, HCR's practical value comes not from the reaction mechanism itself but from the varied functional roles it can assume when combined with DNA nanostructures and ECL. On the basis of recent work, we identify three main functional roles for HCR in ECL biosensing systems.

The first role is as a linear signal amplifier for single target detection. The long, repeated DNA concatemers generated by HCR offer many sites for ECL labels or signal probes to dock, so one recognition event can be converted into many signal outputs. Sun's group used this feature by pairing an Fe single atom catalyst with HCR for myocardial miRNA detection [74]. The target miRNA triggered HCR to produce DNA products with multiple sticky ends. This made it possible for numerous signal probes to attach to the electrode surface, yielding a detection limit of 0.42 fM for miRNA-499 over a linear range from 1 fM to 1 nM.

The second role is as a building block for multi-amplification cascades that overcome the gain ceiling of single HCR. While HCR alone provides linear signal amplification, its gain is inherently limited by the number of hairpin pairs that can polymerize before the chain terminates. To break this ceiling, researchers have developed multiplex HCR cascade strategies in which the product of an initial HCR process serves as the trigger for subsequent HCR cycles. Cao's group constructed an intramolecular donor-acceptor system to solve the efficiency and stability problems of ECL at high excitation potentials, and assembled an ultrasensitive biosensor for miRNA-221 detection using this multiplex HCR cascade [75]. The target-like DNA obtained from the initial HCR process could trigger multiple additional HCR cycles, achieving a detection limit of 5.25 aM. This cascade configuration converts linear signal gain into a quasi-exponential amplification profile without introducing enzymatic steps.

The third role is as an interfacial reconstruction tool for multi-modal and ratiometric sensing. HCR can do more than simple signal amplification. Its assembly of long DNA polymers can be programmed to reshape the sensing interface, allowing several readouts at once or self-referenced quantification. Liao's group built a DNA cube-gated ECL/SERS biosensor for detecting apurinic/apyrimidinic endonuclease 1 (APE1) activity. They combined HCR amplification with a Ti3C2/CsPbBr3@PDA@Au nanocomposite transducer [105]. APE1 cleavage produced an initiator that triggered HCR, which generated long DNA concatemers captured by the cube-tethered hairpin scaffold. This programmed capture brought ferrocene reporters close to Au hotspots, enhancing SERS, while also blocking the interface and suppressing ECL emission. The two signals therefore changed in opposite directions. The ratiometric readout achieved a detection limit of 8.45×10^{-10} U/mL for APE1.

HCR still has inherent limits that narrow its range of use. One limitation is that its linear amplification gives only moderate signal gain when compared with catalytic or enzymatic methods such

as CHA or RCA. Another issue is that the long DNA polymers it produces may undergo incomplete hybridization, premature termination, and nonspecific aggregation, which can hurt reproducibility. A further problem is that the need for carefully designed hairpin probes with low background leakage makes optimization more demanding. These limits have led researchers to explore other or complementary amplification strategies, especially CHA, which we turn to next.

4.1.2. Catalytic hairpin assembly

CHA is the second major class of enzyme-free isothermal amplification. What sets it apart from HCR is that it uses catalytic, or cyclic, signal amplification instead of linear amplification. A typical CHA system contains two hairpin DNA probes, H1 and H2, which are kinetically trapped in metastable states. The target DNA binds specifically to the loop region of H1, causing H1 to unfold. The opened H1 then hybridizes with H2 to form a stable duplex, and the target DNA is released at the same time. The freed target can then start further catalytic cycles. Each cycle produces one H1-H2 duplex, and the target itself is regenerated. Through this target-recycling mechanism, the recognition event is amplified catalytically and no enzyme is involved [106–108]. CHA and HCR use similar components, namely metastable hairpins and strand displacement, but their amplification architectures are fundamentally different. HCR produces one long polymer from each initiator, giving linear gain, while CHA generates many independent duplexes from each target molecule through target recycling, giving catalytic or cyclic gain. The catalytic advantage of CHA comes with stricter probe design requirements and a greater chance of background leakage from spontaneous H1-H2 interactions.

In ECL biosensing, CHA-based amplification has been implemented through three increasingly sophisticated functional modes. The first and most direct mode is CHA as a standalone catalytic amplifier for nucleic acid targets. Wu's group integrated the cascade amplification of CHA circuits and the side branch cleavage activity of CRISPR-Cas12a to construct an ultrasensitive ECL biosensor for pancreatic cancer (PC)-specific tsRNAs with a detection limit of 3.33 fM [78]. Yang's group reported an ECL biosensor for ultrasensitive detection of miRNA-21, and investigated the combination of PdPt@SnS₂NSs nanosheets with CHA, which effectively facilitated the ECL performance with highly sensitive and selective detection of miRNA-21 from 1 aM to 1 nM with a detection limit of 0.02 aM [79].

The second route connects CHA with structural or interfacial engineering to enhance the ECL efficiency. It solves the main problem of CHA. The amplification process is exponential, but converting the amplified products into ECL signals is often restricted by the emitter efficiency, electrode kinetics and accessibility of the probes. Liu's group constructed a ternary ECL system which consisted of high-density DNA nanostructures containing perylene probes and AuAg/N-graphdiyne co-catalysts [109]. In their design, exomiR-155-targeted CHA amplification was realized. Large amounts of products led to probe deposition on the electrode and the detection limit reached 0.49 fM. Clinical experiments demonstrated good agreement with RT-qPCR and the receiver operating characteristic curve analysis indicated excellent diagnostic capability. These results show the practicability of this CHA-based ECL method. Gao's group adopted a similar approach using a DNA microcapsule system. When stimulated by serotonin, it released DNA tetrahedral nanostructures to increase the CHA efficiency owing to its stiffness and valence [110]. Combining the ECL resonance energy transfer between CdS quantum dots and Au nanoparticles, the sensor achieved a detection limit of 0.3 pM for serotonin over six orders of magnitude. This research indicates that programmable DNA nanostructures can serve as both passive

signal carriers and active kinetic regulators. Thus, they help overcome the limitation of reaction efficiency caused by diffusion in traditional CHA systems. Yu's team developed a dual-amplified ECL detecting platform by integrating CHA with rationally designed DNA nanostructures [80]. The target DNA forms I-shaped nanostructures by hybridizing with auxiliary strands, which subsequently activate the following CHA for signal improvement. Li's group also integrated CHA with surface plasmon resonance (SPR) effects, leading to extremely high sensitivity for miRNA-21 detection. In this case, the SPR effect provides extra amplification to the ECL signal beyond that provided by CHA alone [81].

The third mode takes CHA beyond nucleic acid detection and into protein and extracellular vesicle analysis by using aptamers to convert signals. This expansion matters because a large share of clinically relevant biomarkers are proteins, not nucleic acids. Here, aptamer binding to a non-nucleic acid target causes release of a DNA strand. That strand acts as the CHA initiator, so a protein-binding event is turned into an amplifiable nucleic acid signal. Feng's group reported a sensitive ECL platform for tumor exosome detection. It combined magnetic preconcentration, CHA amplification, and a zirconium-based MOF/gold nanocluster composite luminophore [111]. In this platform, CD63 aptamer recognition of exosome surface proteins causes release of auxiliary DNA strands. These strands start CHA cycling on the electrode, which immobilizes composite-labeled signal probes. The platform achieves a linear range of 6.12×10^2 to 6.12×10^7 particles/mL with a detection limit of 187.4 particles/mL, and maintains good accuracy in serum samples.

Looking from mode one to mode three, a clear trend emerges. CHA has moved from a simple nucleic acid amplification tool to a flexible signal conversion module. It can be paired with interfacial engineering, physical signal enhancement, and aptamer-based target recognition. This flexibility makes CHA one of the most adaptable amplification strategies in the ECL biosensing toolbox. Still, CHA has inherent limits that should be recognized. First, its catalytic amplification depends heavily on how efficiently the hairpin probes are kinetically trapped. If H1 or H2 opens spontaneously without target, background signal appears. This leakage can become serious when probes are immobilized on electrode surfaces, where local concentrations are high. Second, CHA needs careful optimization of probe sequences, concentrations, and reaction temperatures. The goal is to establish the needed kinetic asymmetry between the target-initiated pathway and the background pathway. Third, when target sequences are short, the first hybridization step between target and H1 may be inefficient, which lowers the overall amplification gain.

4.2. Enzyme-assisted amplification

RCA is a third major amplification strategy. It differs fundamentally from HCR and CHA in three ways. It depends on an enzyme, it needs a circular DNA template, and it produces long single-stranded products with tandem repeats instead of discrete duplexes or polymers of limited length. In a typical RCA reaction, DNA polymerase extends the primer's 3'-OH terminus continuously. It adds deoxynucleoside triphosphates along a circular template. This generates a long single-stranded DNA product that contains hundreds of tandem repeats complementary to the template sequence [112–115].

However, conventional RCA suffers from operational complexity and limited amplification efficiency, restricting its ECL biosensing applications. Researchers have optimized RCA protocols to enhance performance for ECL integration. For example, using RCA as a high-gain enzymatic amplifier for nucleic acid targets, or the *in situ* synthesis of nanomaterials with ECL activity. Zhang's group

developed a novel DNA cascade amplification comprising dual hairpin DNA recognition-triggered single target recovery (D-STR) and tandem DNA structure-controlled rolled-circle amplification (C-RCA), which acted as signal amplifiers for constructing highly specific and ultrasensitive ECL biosensors for human immunodeficiency virus (HIV) DNA fragment detection (Figure 2) [83]. Not only does it show great potential in avoiding false-positive signals compared to conventional single-target recognition, but it also significantly improves amplification efficiency at the same time, with a detection limit as low as 27.0 aM. Hong's group developed a simplified RCA strategy to drive the *in situ* production of Ag NCs, which resulted in a simpler reaction process compared to conventional RCA and exhibited high ECL signals in the ternary ECL system (AgNCs/S₂O₈²⁻/Cu-MOFs) and enabled sensitive analysis of APE1 [84].

In addition, combining RCA with additional amplification layers or detection modalities to achieve multiplexed or ratiometric readout. This approach meets the need to obtain more comprehensive analytical information beyond single-target quantification. Zeng's group combined hyperbranched rolled loop amplification (HRCA) with vertically ordered mesoporous silica membrane (VMSF) to construct an ECL biosensor for the detection of the acute myeloid leukemia biomarker has-miR-10a-5p, which can detect the target substances in serum samples without complex pre-processing [85]. Zhao's group designed a multifunctional ECL biosensor based on AuNCs/Au-MnO₂ nanoflower RET system combined with RCA for dual-targeted detection of prostate biomarker PSA and miRNA Let-7a [86]. The proposed multifunctional biosensing system is also suitable for the highly sensitive detection of other disease markers, which has broad practical and clinical applications.

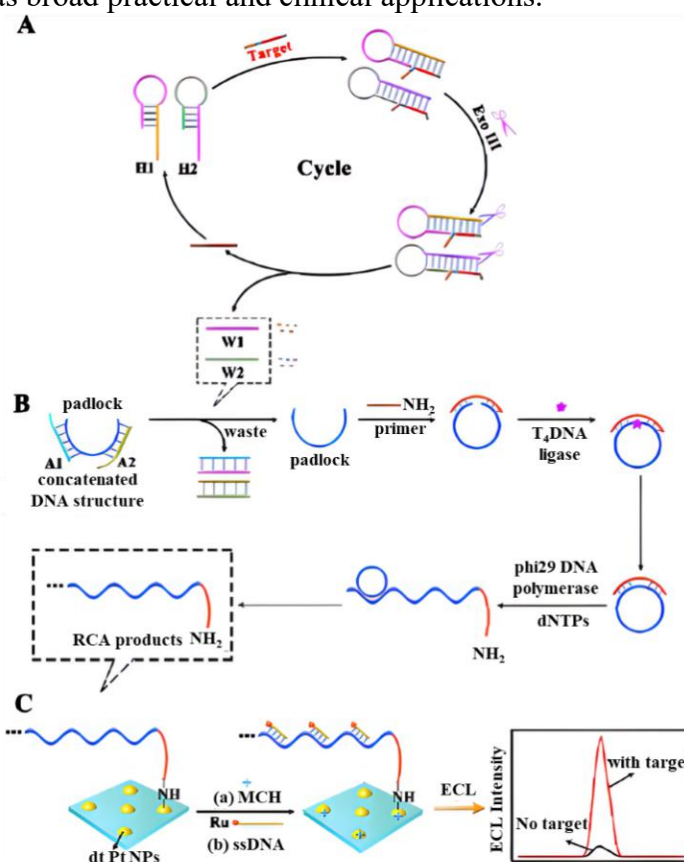


Figure 2. Schematic description of the specific and sensitive biosensor for HIV DNA fragments detection based on (A) double-recognition-triggered single-target recycling and (B) concatenated DNA structure-controlled rolling circle amplification; (C) Construction process of the proposed ECL biosensor [83]. Copyright 2021 American Chemical Society.

4.3. DNA walker systems

DNA walker systems have a distinct way of signal amplification from that of HCR, CHA and RCA. They do not need the formation of nucleic acid polymers or enzymatic replication. Instead, they are like biological nanomachines which transform the biochemical recognition procedures into pre-programmed mechanical movements along a certain route. This movement is mainly supported by strand displacement or enzymatic cleavage. Therefore, it can lead a number of signal probes or catalytic reactions to the particular detection zones one after another. So the signal amplification is based on the spatial concentration instead of the increase in the amount of molecules. A DNA walker system generally includes three elements: a movable strand, a track strand which usually has several binding locations, and a fuel strand or an enzyme which starts the walking of the walker. The walker proceeds along the track one by one. At each step, it cleaves or displaces one binding site and releases a signal marker. Since one walker can deal with many binding sites, the system achieves signal amplification by using a “one walker and multiple substrates” stoichiometry [116–118]. For ECL biosensing, DNA walkers allow the sequential supply of several luminescent substances or reactants to the specified detection areas.

Early DNA walkers were single-footed, moving along a 1D track with relatively slow kinetics and limited processivity. To overcome these limitations, researchers have developed multi-footed and 3D walkers that achieve faster motion and higher substrate processing efficiency through spatial confinement and multivalent interactions. For example, a 3D walker based on space double-confined CHA exhibited walking speeds approximately 8–23 times faster than conventional CHA, and a tetrahedral walker with four arms reached saturation in ~40 min with an initial rate 1.55-fold faster than a fixed swing-arm walker and 4.54-fold faster than a bipedal walker [119,120]. Sun's group proposed a “switching” ECL biosensor based on the excellent ECL properties of MoS₂-GODs nanocomposites combined with DNA walker signal amplification for highly sensitive detection of specific DNA sequences with a detection limit as low as 25.1 aM [87]. Liao's research team has developed an ECL biosensor based on a DNA tetrahedron and a novel, controllable DNA walker with a swing arm. Compared to DNA walkers based on fixed swing arms, this sensor exhibits higher response efficiency, a reaction time reduced by 30 minutes, and higher signal intensity (Figure 3) [88]. The sensor achieved ultra-sensitive detection of miRNA Let-7a with a detection limit as low as 4.92 fM over a wide linear range of 10 fM to 100 nM. Guo's group proposed a crown-shaped DNA walker based on ECL-RET and DNA tetrahedra for the detection of tumor exosomes [89]. This ingenious structure allowed the spatial confinement effect of nanostructures to be improved and the DNA walker exhibited a higher reaction rate. Li's group constructed an ECL resonance energy transfer biosensor for serotonin detection by integrating proximity recognition with a CHA-driven bipedal walking system [121]. Aptamer-functionalized magnetic beads convert target recognition into nucleic acid signal transduction, releasing trigger strands that assemble with two auxiliary sequences to form bipedal proximity probes. The bipedal design accelerates reaction kinetics relative to single-footed counterparts, achieving a detection limit of 0.28 pM across a linear range of 1 pM to 1 μM, and delivers reliable quantification in human serum. This work extends DNA walker-based ECL biosensor to small-molecule neurotransmitter analysis.

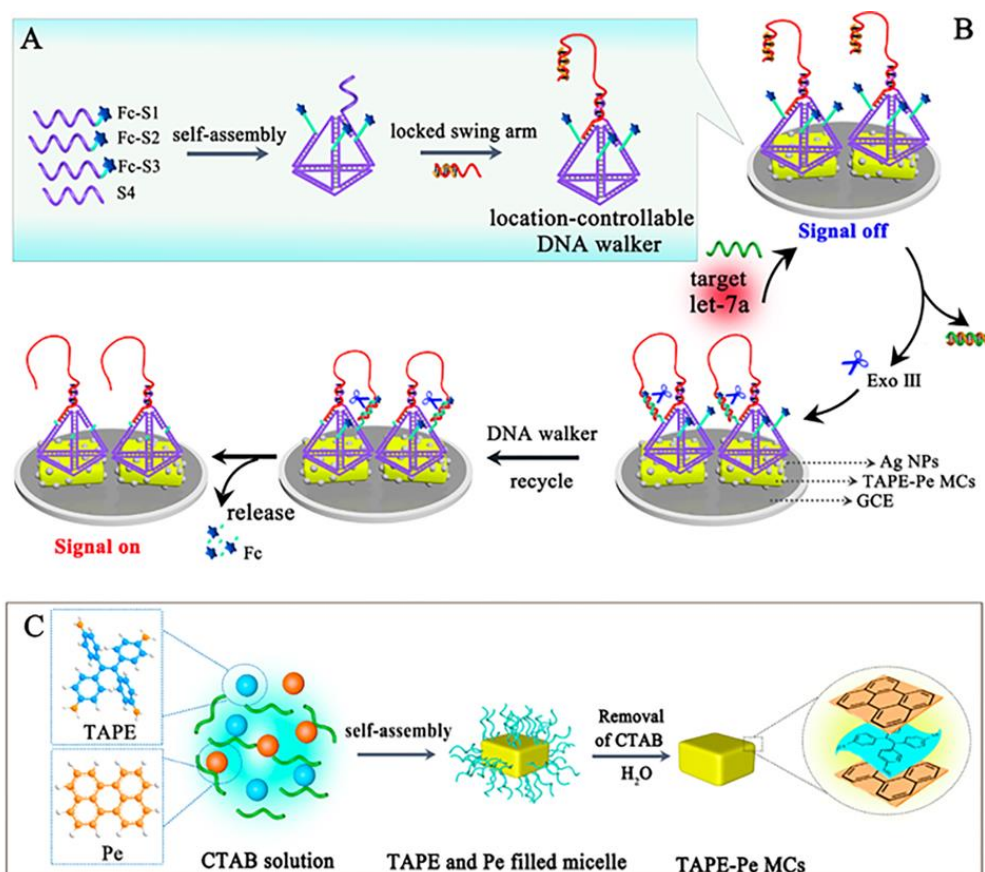


Figure 3. Schematic illustration of (A) the location-controllable DNA walker fabrication; (B) The operating principle of the biosensor based on the location-controllable DNA walker for let-7a determination; and (C) preparation of tape-pe mcs [88]. Copyright 2021 American Chemical Society.

In addition, the integration of DNA walker and signal amplification strategies not only can fully utilize the respective advantages of both, but also has become a popular method in the field of biosensing nowadays. Yang's group achieved ECL ratio detection of amyloid- β oligomers (A β O) by binding a DNA walker to HCR and introducing Ag NPs to the electrode surface [90]. Chen's group prepared Au NCs/Zn-HDS nanocomposites exhibiting 4.9-fold higher ECL emission and 10.9-fold higher ECL efficiency than Au NCs, and combined them with bipedal DNA walker generated by target-induced DNA strand substitution and CHA, which further enhanced the signal intensity and achieved highly sensitive detection of soluble urokinase-type plasminogen activator receptor (suPAR) [91]. Yang's group synthesized a MOF (Dy-TBAPy) with excellent ECL properties, used it as an ECL signaling probe, and combined it with a 3D DNA walker and CHA strategy to construct an ECL biosensor with high sensitivity and specificity, which successfully achieved ultra-sensitive detection of miRNA-21 [92]. Peng's group innovatively developed Sm-MOFs as ECL luminophores and combined them with the triple amplification strategy of HCR, CHA and DNA walker to successfully achieve highly sensitive detection of acetamiprid (ACE) [93].

Walking systems powered by exonucleases have also widened the use of ECL biosensors based on DNA walkers, extending beyond systems driven by strand displacement. Zhang's group built an ECL aptasensor with a DNA walker powered by exonuclease III. It used a covalent triazine framework as the luminophore and targeted carcinoembryonic antigen [122]. Target binding releases complementary DNA strands. These strands hybridize with hairpin probes anchored on bimetallic semiconductive MOF

nanoparticles. Exonuclease III digests the duplexes that form, which triggers cyclic walking. The remaining hairpin fragments bridge the framework nanoparticles to the electrode interface. The bimetallic framework accelerates co-reactant decomposition and electron transfer, significantly amplifying the ECL response. This biosensor achieves a detection limit of 2.91 fg/mL with a linear range of 1.00– 5.00×10^4 pg/mL, and delivers reliable quantification in human serum samples. Shi's group developed an ECL biosensor for okadaic acid detection using a terbium metal-organic gel as the luminophore paired with an Exo III-driven cyclic DNA walker [123]. The sensor achieves a detection limit of 0.16 pg/mL across a linear range of 1–1000 pg/mL, with excellent recovery rates in shellfish matrices.

These signal amplification strategies based on DNA nanostructures play an important role in ECL biosensing. Each has its own strengths and suitable applications. Together they offer diverse options for achieving highly sensitive and specific bio-detection.

4.4. DNAzyme-based amplification

DNAzymes are catalytic DNA molecules [124] whose integration into ECL biosensing systems enables functional expansion and performance enhancement. DNAzyme-integrated systems typically employ sequence-specific catalysis to trigger chemical cascades modulating ECL signals. Xie's group reported an optically triggered DNAzyme platform for “on-demand” activation of ECL c-myc RNA analysis, and this ECL biosensor can effectively restrict the ECL signal to the target mRNA, which can effectively avoid false positive signals [94]. Sun's group designed an ECL biosensor combining DNAzyme with a RET system to detect myocardial miRNA, providing a sensitive and reliable method for myocardial miRNA detection [125]. Chen's group reported a novel metal-organogel Eu-MOGs combined with DNAzyme-mediated dual-drive DNA walker for an ultrasensitive ECL biosensor to detect miRNA-222 [126]. In addition, DNAzyme walkers have been also applied in ECL biosensor through interfacial reaction rate regulation. Shao's group reported a homogeneous ECL biosensor for copper ion detection based on a Cu^{2+} -activated DNAzyme walker that modulates the surface properties of ECL nanoprobe [95]. Target copper ions activate the DNAzyme to cyclically cleave substrate strands, reducing surface negative charge and steric hindrance of the nanoprobe and enhancing their accessibility to the electrode and co-reactant. This signal-on homogeneous strategy achieves a detection limit of 0.025 nM across a wide linear range of 0.1 nM to 10 mM, and has been validated in food and environmental matrices. The homogeneous design eliminates complex electrode modification steps, providing a convenient one-step detection paradigm.

The integration of DNAzyme with emerging ECL emitters has further expanded the functionality of biosensing platforms. Zeng's group employed environment-friendly MnO_2 quantum dots as highly efficient ECL emitters, and developed a DNAzyme-induced self-feedback DNA amplification strategy that converted trace miRNA-222 into abundant output DNA through cyclic target analogue regeneration [96]. The AgNPs served as coreaction accelerators to enhance the MnO_2 QDs/ $\text{S}_2\text{O}_8^{2-}$ system, achieving a detection limit of 12.90 aM for liver cancer marker miRNA-222. Geng's group covalently grafted the AIEgen TCBPE onto UiO-66- NH_2 through amide bonding, creating a nanoconfined environment that suppressed intramolecular motions and reduced nonradiative decay [97]. A target-triggered three-legged DNAzyme walker system catalytically cleaved methylene blue-labeled hairpin probes on the electrode surface in the presence of Mg^{2+} , simultaneously restoring ECL emission via ECL-RET disruption and

attenuating the electrochemical signal. This dual-mode strategy achieved detection limits of 3.27 fg/mL (ECL) and 6.76 fg/mL (EC) for 17 β -estradiol.

In view of the integrating and consolidating role of the DNAzyme system, the preparation of biosensors with multi-modal and multi-signal has become a research hotspot. Lu's group developed a self-powered DNAzyme walker fluorescence (FL) and ECL dual-mode imaging platform to achieve accurate detection of SARS-CoV-2 spiking proteins at the single-molecule level [127]. Specific activation of the DNAzyme walker by the target proteins powered the continuous operation of the system, enabling the simultaneous detection of both FL and ECL signals. The dual mode significantly improves the accuracy of detection of spiny proteins compared to single signal output. Yi's group developed a DNAzyme-driven dissipative cycling system for single-nucleotide variant detection, which generated transient fluorescence signals for real-time monitoring while simultaneously recording cumulative ECL signals via bipolar electrode-based signal transduction [128]. This dual-signal modality, combined with a depletion strategy to enhance mutation discrimination, achieved selective detection of 0.01% variant allele frequency against wild-type backgrounds.

4.5. CRISPR-Cas-integrated cascade amplification strategies

A key distinction is that CRISPR-Cas systems work differently, mechanistically, from the nucleic acid amplification strategies described in Sections 4.1 to 4.3. HCR, CHA, and RCA raise signal through programmed nucleic acid assembly or replication. CRISPR-Cas, in contrast, serves primarily as a programmable recognition and cleavage module. It does not amplify the target nucleic acid itself. However, in certain Cas effectors, target activation causes trans-cleavage, so a single recognition event can be turned into multiple cleavage events. This offers enzymatic signal amplification. As a result, CRISPR-Cas-integrated ECL biosensors are better viewed as hybrid cascade architectures. In these designs, CRISPR-Cas provides sequence-specific recognition and trans-cleavage-mediated signal conversion or amplification, while HCR, CHA, or RCA contributes an additional nucleic acid amplification stage [129,130].

In these cascade ECL biosensing systems, the CRISPR-Cas module carries out two main functions. Firstly, it identifies the target nucleic acid and subsequently sends the signal. When the target nucleic acid is detected, the active Cas protein cuts the nearby nucleic acid substrates, leading to the release of many trigger strands. These trigger strands can activate the subsequent DNA amplification processes like HCR, CHA or RCA. Therefore, one target recognition event can produce multiple amplifiable nucleic acid signals which are beneficial in reducing the false positive signals caused by non-specific amplification. Wang's group manufactured a dual-mode biosensor using HRP/Au NPs nanocomposites and CRISPR/Cas12a [82]. The activation of Cas12a cleavage by the target results in the degradation of HRP/Au NPs probes. By means of magnetic separation, the supernatant containing HRP is collected to enhance the anodic ECL effect and the TMB chromogenic reaction provides visual detection. Luo's group designed a three-stage cascade amplification platform based on T7 transcription, CRISPR-Cas13a collateral cleavage and HCR for the sensitive detection of α -synuclein oligomers, an important marker of Parkinson's disease [98]. The recognition of the target by a conformation-changing aptamer reveals an embedded T7 promoter sequence which initiates *in vitro* transcription to produce a large number of RNA transcripts. These RNAs activate Cas13a to cleave the hairpin substrates and release DNA initiators, forming surface-immobilized HCR and generating long DNA concatemers with many ECL labels.

Through triple signal amplification, the biosensor achieves a detection limit of 8.3 aM with recovery rates between 97.0% and 104.3% in diluted human serum. This modular design expands the CRISPR-DNA cascade systems to non-nucleic acid targets by applying aptamer-mediated signal conversion. He's group constructed a simple one-step ECL biosensing system which combines RCA-induced CRISPR/Cas12a with magnetic bead-based quenched ECL probes [99]. The initiation of RCA by the target produces long amplicons which activate Cas12a at the same time, making full use of both cis-cleavage for template reuse and trans-cleavage for the degradation of single-stranded DNA bound to $\text{Ru}(\text{bpy})_3^{2+}$ -loaded magnetic beads. The unified one-step format eliminates the need for multiple incubation steps and simplifies the experimental procedures. The combination of RCA and CRISPR-Cas systems has also been used in branched RCA formats to improve the signal. Zheng's group developed a homogeneous ECL biosensor for detecting N-nitrosodimethylamine (NDMA) by connecting RCA, CRISPR/Cas12a-driven HRCA and a mesoporous silica film-modified electrode [100]. The CRISPR/Cas12a system effectively eliminated primer dimer-induced false positives through its cis- and trans-cleavage activities. The triple amplification strategy achieved a detection limit of 5.33 pg/mL for NDMA, demonstrating the versatility of RCA-CRISPR cascades for small-molecule detection.

Second, DNA amplification circuits are employed to pre-amplify target sequences, thereby activating more Cas effector proteins and establishing a positive feedback loop that links target amplification, Cas activation and ECL signal output. Li's group designed a signal-on ECL resonance energy transfer biosensor for miR-543 detection based on CRISPR-Cas13a and magnetic separation, where target-induced Cas13a activation cleaved RNA motifs to release gold nanorods from magnetic beads, reducing energy transfer efficiency and recovering ECL intensity [101].

Generally, the cascade amplification using CRISPR-Cas is regarded as a hybrid structure rather than a simple amplification procedure. The overall amplification factor mainly depends on the CRISPR-associated trans-cleavage cycle and frequently on the coupled nucleic acid amplification systems such as HCR, CHA or RCA. CRISPR-Cas offers the function of specific target recognition.

The cascade amplification can improve the sensitivity; however, the number of amplification steps will lead to some practical problems. Each extra step, whether it is an enzymatic digestion, strand displacement or Cas-mediated cleavage, may cause background interference, increase the testing time and enlarge the experimental variability. For instance, the hairpin probes in CHA may open spontaneously, HCR might occur in an unspecific polymerization and the Cas effectors may exhibit off-target cleavage. All these may result in false positive signals, which can be further amplified in the following cascades. Multi-step procedures also increase the chance of contamination, need careful control of the temperature and reagents and are difficult to apply in the point-of-care method. Therefore, the analytical accuracy of a cascade system is not decided only by its LOD. It also relates to the signal-to-background ratio, reproducibility, convenience of operation and resistance to complex environments. In the actual biosensor application, a moderate amplification with low background noise and good reproducibility is usually more important than a very low LOD obtained by a fragile multi-step cascade. To overcome these issues, one-pot reactions or integrated systems, high-fidelity recognition modules such as CRISPR-Cas to regulate the signal production, ratiometric or dual-signal self-calibration and confinement of unspecific interactions in DNA frameworks can be utilized. Thus, when comparing various amplification methods, the sensitivity should be balanced with the background level, the assay duration and the reproducibility.

instead of being assessed merely by LOD. So, the complexity of the amplification strategy should be reasonably adapted to the intended use.

5. Biosensing applications

ECL biosensors based on DNA nanostructures have been widely studied for the identification of different kinds of biological and chemical substances. Their capability to be arranged in structure and offer several channels for signal enhancement enables the formation of sensitive and specific detecting systems. In this paper, we introduce four main application areas: nucleic acids; proteins and disease-related biomarkers; small molecules and metal ions; and intracellular markers. Furthermore, we explain the building of DNA nanostructures according to the different needs of various targets.

5.1. Nucleic acid detection

Nucleic acid detection is the leading field of application for ECL biosensors based on DNA nanostructures [131]. This predominance is not due to chance. The Watson-Crick base pairing that joins the DNA nanostructures is the same mechanism which permits them to capture the target nucleic acids, so the design of the scaffold and the recognition of the analyte work in harmony. Moreover, the DNA sequences can be programmed, which allows the direct construction of amplification and transduction modules into the recognition structure. Hence, there is no necessity for an intermediate signal conversion process. In this area, three topics have been paid special attention: the detection of pathogen nucleic acids, the analysis of microRNAs and the detection of circulating tumor DNA.

5.1.1. Pathogen nucleic acid detection

Detecting the nucleic acids from pathogens needs high sensitivity. At the initial stage of infection, the pathogen amount is generally small, so the test must detect the few remaining targets. It should give quick results to allow early clinical treatments [132,133]. The DNA tetrahedral nanostructures have been found to be beneficial for this purpose [32,134]. Their stable 3D shape retains the capture probes in solution with proper orientation and distance. This improves the hybridization efficiency towards the target nucleic acids and also aids in reducing the non-specific adsorption from the complex clinical samples like serum, saliva and nasopharyngeal swab extracts.

A representative demonstration comes from Fan's group, who constructed an ECL biosensor targeting the SARS-CoV-2 RdRp gene via entropy-driven amplification on a DNA tetrahedron-modified electrode [135]. The tetrahedral scaffold provides well-ordered interfacial architecture to improve collision efficiency between target sequences and recognition probes. The entropy-driven amplification circuit relies on toehold-mediated strand displacement and requires no enzymes, converting each target-recognition event into multiple signal outputs. This sensor reached a detection limit of 2.67 fM within the linear range of 1 fM to 100 pM. The result illustrates that combined structural ordering and programmable amplification can yield clinically relevant sensitivity without thermal-cycling-associated complexity.

The integration of DNA tetrahedra with CRISPR-Cas systems has further expanded the capabilities of pathogen nucleic acid detection. CRISPR-Cas effectors, particularly Cas12a and Cas13a, possess two features highly complementary to DNA nanostructure-based sensing. These include target-activated collateral cleavage activity, which amplifies signals by degrading multiple reporter probes, and

programmable specificity for single-nucleotide variant discrimination. Zhang's group developed a CRISPR/Cas12a-mediated ECL biosensor for SARS-CoV-2 nucleic acid detection using pH-engineered DNA tetrahedra [136]. The tetrahedral design avoids direct immobilization of recognition probes on electrode surfaces. Direct immobilization frequently impairs probe activity via random orientation and surface crowding. Instead, the system adopts a three-stranded nucleic acid assembly with strong pH dependence. This pH-responsive assembly enables highly reproducible sensor fabrication. More importantly, sensor regeneration can be realized merely by adjusting buffer conditions, which resolves a major practical drawback of disposable ECL sensors. Coupled with the trans-cleavage activity of Cas12a, the biosensor achieved a detection limit of 43.7 aM toward SARS-CoV-2 targets.

For bacterial pathogen detection, Zhang's group constructed an amplification-free ECL biosensor for *Fusobacterium nucleatum*, a key pathogen linked to colorectal cancer and periodontal disease. The sensor employed tetrahedral DNA probes conjugated with ferrocene quenchers on a coralliform gold-functionalized electrode [137]. Relative to conventional linear or planar probe configurations, the rigid tetrahedral scaffold greatly improved probe accessibility and cleavage efficiency by Cas12a. Benefiting from high ECL readout sensitivity and boosted Cas12a activity on the tetrahedral scaffold, this amplification-free format reached a detection limit of 1 CFU/mL for the bacterium, with a linear range of 10 fM to 100 nM for the *fadA* gene. This work demonstrates an essential design principle. DNA nanostructures offset the lack of pre-amplification by optimizing the efficiency of CRISPR-based signal transduction, which simplifies sensor workflows while preserving ultra-high sensitivity.

5.1.2. MicroRNA detection

MicroRNAs have been paid much interest as diagnostic indicators. They are resistant to changes in body fluids and exhibit tissue-specific expressions, becoming abnormal in certain diseases like cancer, cardiovascular disorders and neurodegenerative diseases [138,139]. However, the detection of miRNAs is more difficult than that of longer nucleic acid targets in two aspects. Firstly, their short length, approximately 19 to 25 nucleotides, does not facilitate the acquisition of suitable annealing conditions to ensure high specificity due to the decreased hybridization energy between the probe and the target. Secondly, there is a high degree of sequence similarity among miRNA families. The members may only differ by one nucleotide, therefore the test must be able to distinguish them clearly to avoid cross-reaction [140].

DNA tetrahedral scaffolds address the first problem by pushing capture probes out into solution, which makes targets easier to reach. Wang's group built a noncovalent spherical nuclease system for detecting miRNAs linked to myocardial infarction, with probes anchored on DNA tetrahedra [141]. The tetrahedral scaffold places recognition probes at defined spacing and orientation. It also lowers steric hindrance that would otherwise block hybridization between short miRNA sequences and their complementary probes. This sensor achieved a detection limit of 33 aM for miRNA-499. These results show that structural ordering provided by tetrahedral scaffolds can get around the thermodynamic disadvantages of hybridizing short sequences. Peng's group used a DNA tetrahedron as a stable scaffold. It anchored locked walker strands and ferrocene-labeled signaling strands, which allowed dual swing arms to be activated at the same time for miRNA-24-3p detection (Figure 4) [142]. The tetrahedral scaffold tuned lateral probe spacing at the sensing interface, accelerated probe-target binding kinetics, and precisely controlled probe orientation and density. Coupled with $\text{Ru}(\text{bpy})_3^{2+}@\text{AuNPs}@\text{SWCNTs}$

as the ECL emitter, this design yielded a detection limit of 60 aM. Benefiting from the spatial organization of the tetrahedral scaffold, the dual-swing-arm setup improved walking kinetics relative to single-arm configurations. This finding indicates structural rigidity improves not only passive target capture but also active signal amplification.

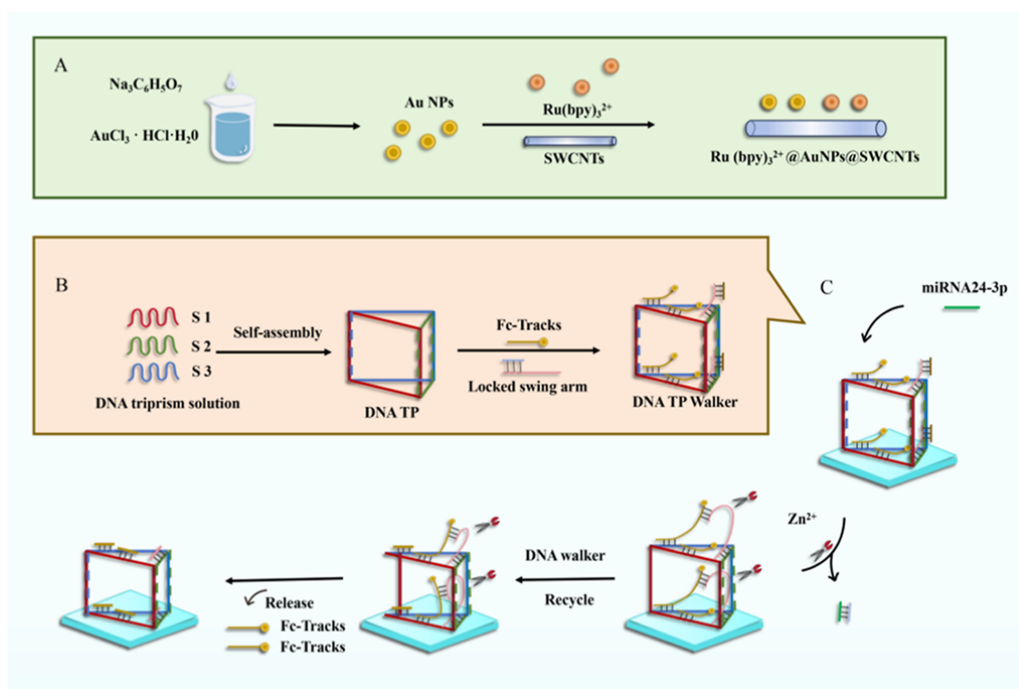


Figure 4. Schematic diagram of (A) luminescent material synthesis; (B) DNA walker preparation; and (C) ECL biosensor assembly [142]. Copyright 2025 American Chemical Society.

Two complementary strategies have been developed for multiplexed miRNA detection in one assay, namely wavelength-resolved detection and DNA logic circuits. Yin's group prepared a multifunctional Zr-MOF@PEI@AuAg nanocluster nanocomposite, which exhibited intense, stable dual-wavelength ECL emission at 535 nm and 644 nm [143]. DNA tetrahedra carrying two different quenching probes BHQ1 and BHQ3 were immobilized onto composite-modified electrodes. The introduction of miRNA-141 and miRNA-155 induced differential quenching toward the two ECL wavelengths via resonance energy transfer. Such behavior permitted simultaneous detection of the two targets, with detection limits of 19 aM and 26 aM respectively. This wavelength-resolved approach represents a universal detection scheme. It can be adapted for alternative miRNA targets merely by replacing recognition sequences on tetrahedron vertices.

DNA logic circuits provide an alternative strategy for multi-miRNA detection with improved diagnostic specificity. Wavelength-resolved detection separately quantifies individual target concentrations. By contrast, logic-gated sensors produce positive outputs only upon co-existence of multiple biomarkers, which lowers false-positive signals caused by elevated single biomarkers under non-pathological conditions. Zhu's group built an ECL cascade amplification platform for dual acute myocardial-infarction-related miRNA detection based on an AND-gate logic circuit [144]. miRNA-133a-triggered DNAzyme cleavage and miRNA-499-triggered duplex-specific nuclease cleavage separately yield components for fuel hairpin assembly and initiator strand generation. HCR amplification proceeds and generates the final ECL signal only when both miRNAs are available. This AND-gate setup guarantees sensor output

corresponds to concurrent upregulation of two disease-relevant miRNAs, hence enhancing diagnostic accuracy compared with single-target assays. Han's group further developed a dual-miRNA detection platform by combining DNA logic gates with ECL readout, where DNA hexahedra served as capture probes [145]. The platform implements AND and OR logic gates for miRNA-328 and miRNA-499 detection. Sensor functions can be adjusted to satisfy distinct diagnostic requirements according to clinical scenarios. Ultrasensitive detection of the two miRNAs was achieved, with detection limits of 3.1 fM for the AND configuration and 1.7 fM for the OR configuration. This result validates the flexibility of DNA-nanostructure-enabled logic gating for multiplexed miRNA analysis.

Combining DNA logic circuits with ML has further improved diagnostic performance in miRNA detection. Zhang's group reported a dual-target ECL biosensor aimed at bladder cancer-related miR-183-5p and miR-96-5p. It merged a DNA AND logic gate with toehold-mediated strand-displacement circuits [146]. Quantified miRNA levels from urine samples were imported into a ML-driven logistic regression model. This model reached 100 % accuracy when distinguishing bladder-cancer patients from healthy controls. Zhang's group constructed an allosteric-DNAzyme-based ECL biosensor for acute myocardial infarction diagnosis. YES, AND, OR, and INHIBIT logic gates were implemented within one unified molecular framework [147]. In a similar way, miRNA concentrations measured in patient serum samples were input into another logistic regression ML model. This model achieved 100% classification accuracy in distinguishing AMI patients from healthy individuals. This kind of combination, linking DNA computing, ECL transduction, and data-driven analysis, opens a new paradigm for intelligent molecular diagnostics. Rather than giving only a simple positive or negative readout, the sensor supplies quantitative inputs for clinical decision-making algorithms.

5.1.3. Circulating tumor DNA and other nucleic acid targets

Circulating tumor DNA has become a minimally invasive biomarker for cancer detection and monitoring. Unlike pathogen nucleic acids and miRNAs, ctDNA is present at extremely low levels in blood. It usually makes up parts per million of total cell-free DNA [148,149]. It often carries point mutations, which must be clearly distinguished from wild-type sequences. Pan's group built an ECL biosensor for ctDNA detection. Al-doped CuS colloidal nanocrystals served as the ECL emitter. The work used multiple HCR products, and target-triggered degradation of those products improved response sensitivity. The detection limit reached 2.74 aM [76]. Linear DNA nanostructures formed during amplification allowed ECL labels to be loaded at high density onto HCR products, giving considerable signal gain. At the same time, the inherent specificity of target-induced degradation ensured effective discrimination of non-target sequences.

DNA methylation is a key epigenetic biomarker for cancer diagnosis, and it offers a representative example of this strategy. Wang's group reported an intramolecular ECL resonance energy transfer biosensor for methylated DNA detection. It was based on Y-shaped DNA nanostructures [150]. In this design, methylated target DNA hybridizes with hairpin DNA and glucose oxidase-labeled auxiliary DNA, forming Y-shaped nanostructures. In the construct, GOD is placed at one end of the Y-shaped architecture, while the luminescent material sits at the other end. This allows precise tuning of the distance between ECL donors and acceptors. The GOD-catalyzed enzymatic reaction produces hydrogen peroxide, which modulates ECL intensity through a combination of energy transfer and enzymatic signal

amplification. The sensor achieves sensitive methylated DNA detection, with a detection limit of 65 aM over a linear range from 100 aM to 20 pM.

For multiplexed detection of disease biomarkers, Wu's group constructed an ECL biosensor for simultaneous detection of hepatitis A virus and hepatitis C virus by adopting Y-shaped DNA structures with spatial-potential resolution [151]. In channel A, HAV recognition drives hybridization between Ag-nanoparticle-labeled quenching probes and Y-shaped DNA. These probes couple with donor MOF and reduce positive-potential signals via ECL-RET. In channel B, HCV-triggered hybridization onto Y-shaped DNA introduces CdSe quantum dots into Ce-MOF and elevates negative-potential signals. Taking advantage of the spatial layout of Y-shaped scaffolds and potential-dependent ECL emission from distinct luminophores, the sensor achieves parallel detection of HAV and HCV. Their corresponding detection limits are 50 fM and 0.2 pM. This work proves that multi-arm topology of Y-shaped DNA is applicable not only to single-target sensing but also to multiplexed analysis by establishing orthogonal signal transduction channels.

5.2. Protein detection

Protein biomarkers are important for the clinical diagnosis. Nevertheless, the detection of Protein biomarkers using ECL is different from that of nucleic acid targets. One should keep their binding conformation before immobilizing antibodies or aptamers on the electrode. Besides, the recognition process needs to be changed into a measurable signal which can be increased. DNA nanostructures can fulfill both these requirements. The stable frameworks can place the capture antibodies or aptamers so that they are always available, and the programmable DNA circuits can transform the protein binding into a series of nucleic acid amplifications.

Aptamers can specifically bind to certain protein targets with high affinity and specificity. They serve as a direct molecular connection between DNA nanostructures and protein analytes. When aptamers are included in the DNA nanostructure frameworks, the structure controls the orientation, spacing and multivalency accurately, and aptamers make use of these features [152].

Xiong's group constructed a Y-shaped DNA structure for mucin 1 (MUC1) detection. MUC1 is a transmembrane protein overexpressed in epithelial cancers [153]. Target-triggered conformational changes of the Y-shaped scaffold tune the distance between the ECL donor (TPE-UiO-66) and acceptors (Au nanoparticles and doxorubicin). Without MUC1, the Y-shaped conformation keeps donors and acceptors spatially separated. After MUC1 binding, the aptamer-carrying arm rearranges spatially, bringing donors and acceptors into close proximity. This process initiates ECL resonance energy transfer and generates strong ECL signals. Benefiting from programmable geometry of the Y-shaped scaffold, this "turn-on" sensing mechanism reached a detection limit of 7.6 fg/mL for MUC1 within the linear range from 10 fg/mL to 1 ng/mL.

Ling's group rationally designed assembled Y-type DNA nanostructure-spherical nucleic acids to work as target transducers. This system achieved quantitative activation of CRISPR-Cas12a toward α -methylacyl-CoA racemase (AMACR), a prostate-specific biomarker [49]. The Y-SNA structure contains three functional domains. One is an aptamer for AMACR recognition. Another is a linker segment for assembly. The third is a CRISPR-activating sequence that remains inactive before target-induced conformational switching. When AMACR is recognized, the Y-SNA undergoes structural rearrangement, exposing the CRISPR activator. As a result, Cas12a is activated and induces trans-cleavage of reporter

probes, producing ECL output. Compared with conventional aptamer-based sensors, the Y-SNA design offers two main advantages. Multivalent aptamer display on the Y-shaped scaffold improves binding affinity through avidity effects. In addition, CRISPR-mediated signal transduction converts protein-recognition events into amplified nucleic-acid signals. The sensor enables quantitative AMACR detection from 10 ng/mL to 100 µg/mL, with a detection limit of 1.25 ng/mL.

Beyond aptamer-based strategies, DNA nanostructures also serve as engineered interfacial scaffolds for antibody-based ECL immunosensing, where the central challenge is to immobilize capture antibodies with controlled orientation and retained activity while minimizing nonspecific adsorption from clinical matrices. Zhang's group developed an ultrasensitive ECL immunosensor for the bladder-cancer urinary biomarker human complement factor H-related protein 1 (CFHR1) by combining a DNA tetrahedral capture interface with a metal-organic framework signal probe [35]. In this design, capture antibody Ab1 was anchored on a three-dimensional TDN scaffold preassembled on a gold nanoparticle-deposited electrode (TDN-Ab1); the rigid tetrahedral framework projects the antibodies into solution, improving the accessibility of CFHR1 to the probes and reducing surface crowding. The sandwich complex was then labeled with a signal probe prepared by *in situ* growth of AuNPs on Ru(bpy)₃²⁺-loaded MOF nanospheres (Ru-MOF@AuNPs-Ab2), in which the nanoconfined MOF pores immobilize the luminophore against aggregation-induced quenching and the AuNPs accelerate electron transfer, jointly affording a 2.5-fold ECL enhancement over Ru-MOF alone. The immunosensor exhibited a linear range of 0.1 fg/mL to 10 pg/mL with a detection limit of 0.069 fg/mL, together with good reproducibility, stability, and selectivity against interferents at tenfold excess concentrations. This work exemplifies the dual role of DNA nanostructures in immunosensing: TDNs optimize the biorecognition interface, while nanostructured signal carriers provide ECL enhancement, representing a design principle that may be extended to other clinically relevant protein biomarkers.

DNA nanostructures are used for the detection of proteins as well as to observe the conformational changes produced by proteins which may suggest the disease state. Liu's group has developed a ratiometric electrochemical/ECL biosensor to detect amyloid-β oligomers, an important indicator of Alzheimer's disease [154]. In this biosensor, an upstream entropy-driven DNA circuit is connected to a downstream HCR. The magnetic beads coated with aptamers bind to the target oligomers, and the binding of these beads releases the trigger strands. These trigger strands activate the entropy-driven circuit and show the initiation sequences for the following HCR. Because this process can be controlled step by step, it allows for repeated amplifications and increases the fixation of ferrocene labels on the electrode surface. The biosensor can recognize amyloid-β oligomers with a detection limit of 14 fM and can measure the concentrations of the targets in the clinical serum samples from Alzheimer's disease patients. The experimental results agree with those obtained from the ELISA tests.

5.3. Small molecules and metal ions detection

Small molecules and metal ions pose special problems for ECL biosensing that relies on DNA nanostructures. Nucleic acids and proteins have many binding sites and large contact surfaces, so multivalent interactions are easy. Small molecules, by contrast, typically have only one binding epitope. That limits signal amplification that depends on target triggered conformational switching or multivalent binding. Small molecule targets also often lack enough molecular complexity for direct recognition

mediated by nucleic acids. As a result, aptamers or other recognition units that change conformation in response to the target are needed.

Aflatoxin B1 (AFB1) represents a highly carcinogenic mycotoxin that contaminates food and feed. Sensitive low-concentration detection is essential for food-safety guarantee. Li's group constructed an ECL biosensor for AFB1 detection. DNA tetrahedra loaded with nitrogen-doped carbon dots acted as the signal-amplification platform [155]. The tetrahedral scaffold did two main jobs. It created rigid, well ordered interfacial structures that reduced nonspecific adsorption from complex food matrices. It also allowed high density immobilization of N-CD signal probes, which give strong ECL emission after target recognition. Aptamer based AFB1 binding causes conformational rearrangement of the tetrahedral assembly. This changes the distance between N-CD emitters and electrode surfaces. The sensor reached a detection limit of 77 pg/mL over a linear range from 100 pg/mL to 10 µg/mL. Yang's group introduced a defective protospacer adjacent motif locking strategy for AFB1 detection [156]. This PAM lock mechanism strongly suppressed off target background leakage without sacrificing target induced signal amplification. When coupled with magnetic ECL nanoprobe whose surface steric hindrance was controlled by Cas12a cleavage, the homogeneous aptasensor achieved a detection limit of 0.2 pg/mL for AFB1, with a linear range covering six orders of magnitude.

Fan's group designed a dendritic DNA nanostructure via a hairpin-free nonlinear HCR, which assembled numerous CdSe@ZnS quantum dots to form a highly amplified ECL probe [157]. Combined with a Hg²⁺-induced Y-shaped DNA structure that enhanced endonuclease cleavage efficiency through Exo-III and Nt.BbvCI dual-enzyme cycling, this strategy achieved a detection limit of 23.6 fM for Hg²⁺. The dendritic DNA architecture enabled multi-signal output through dense QD loading, demonstrating the power of branched DNA nanostructures in ECL signal amplification. Zhu's group constructed a "signal-off" ECL biosensor system based on versatile and sensitive quantum dots (QDs) [158]. The sensor relies on DNAzyme and CHA amplification strategies. After cascade amplification, numerous ferrocene-labeled Y-shaped DNA complexes were generated on the surface of the QDs-modified electrodes. The developed programmable "signal-off" ECL biosensor platform exhibited excellent sensitivity and detection limits of 35.00 aM, 3.71 fM, and 0.28 pM for target DNA, aptamer substrates (ATP as a model), and ions (Ag⁺ as a model).

5.4. Intracellular marker detection

Biomarker analysis at the cellular level stands at the research frontier of DNA-nanostructure-based ECL biosensing. This field comprises two complementary modes. The first is *in situ* intracellular sensing, in which DNA nanostructures are delivered across cell membranes, retain their structural integrity inside cells, and generate readable ECL responses associated with intracellular targets or the cellular microenvironment. The second is lysate-based intracellular marker analysis, in which nucleic acids or proteins are extracted from cells and quantified by ECL biosensors that exploit DNA-nanostructure-assisted amplification; although this mode sacrifices spatial information, it places fewer demands on intracellular delivery and biocompatibility and therefore accommodates more sophisticated amplification circuits.

In situ intracellular ECL sensing requires DNA nanostructures to survive the delivery process, evade nuclease degradation, and remain functionally accessible in the crowded intracellular environment. In addition, efficient electrochemical excitation and optical readout within intact living cells remain major technical challenges. For nucleic acid targets inside cells, Kong's group fabricated core-brush 3D DNA

nanomachines through rolling circle amplification for ultrasensitive intracellular miRNA-21 imaging (Figure 5) [159]. This core-brush structure consists of a central DNA core surrounded by densely packed brush-shaped DNA strands. After interacting with miRNA-21, the 3D nanomachine triggers autonomous and continuous movement of DNA walking arms to cleave hairpin substrates. It reaches a detection limit of 3.57 aM and completes live-cell imaging within 15 minutes. The 3D framework raises local concentrations of reaction components and shortens the diffusion-driven searching distance for walking arms, greatly accelerating reaction kinetics relative to 1D and 2D walker systems. Notably, the compact and nuclease-resistant core-brush configuration facilitates cellular internalization without transfection reagents. This finding validates the application prospect of DNA nanostructures for direct intracellular analysis. Wu's group further extended HCR to a bipolar electrode-ECL imaging chip for simultaneous detection of miRNA-21 and miRNA-155 in single cells, where the integrated evaluation of two miRNAs effectively eliminated false signals and improved detection accuracy by 10% [77]. This demonstrates that HCR can be coupled with rigid DNA frameworks to achieve causality-locked signal transduction and spatially resolved multiplexed analysis.

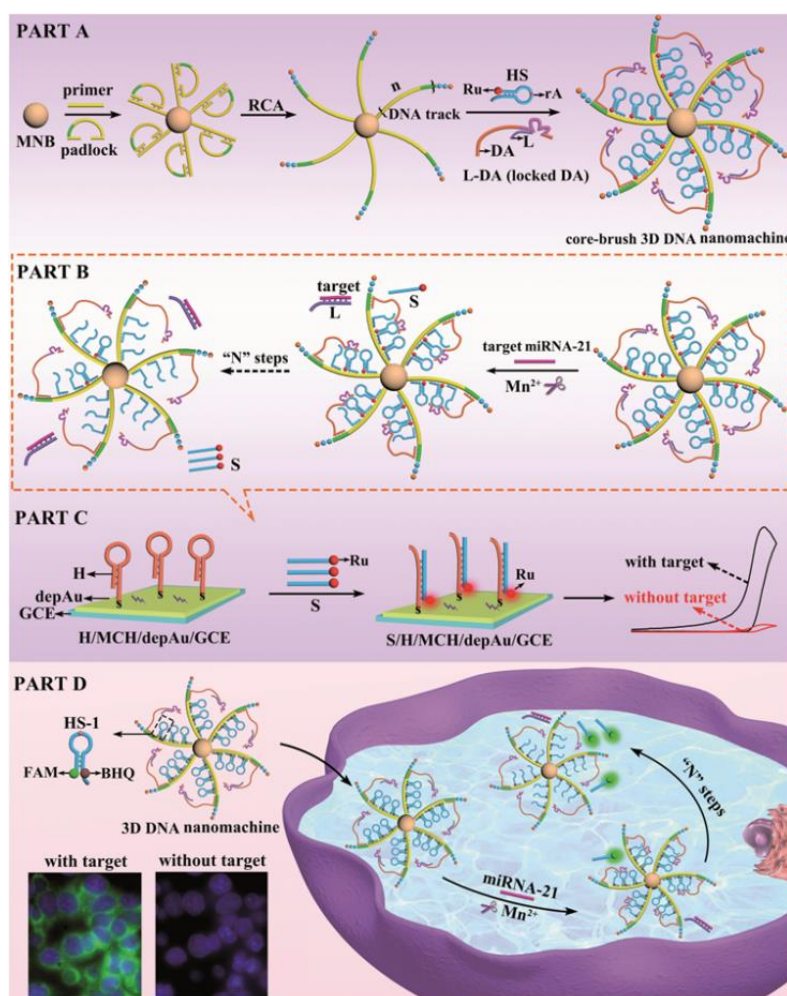


Figure 5. Schematic illustration of (A) the preparation of the core-brush 3D DNA nanomachine; (B) Target miRNA-21 induced recycle cleavage process; (C) The mechanisms of the ECL biosensing system for miRNA-21 sensitive detection; and (D) rapid intracellular imaging of miRNA-21 based on the core-brush 3D DNA nanomachine [159]. Copyright 2021 Royal Society of Chemistry.

For intracellular small molecules, Liu's group developed a stimuli-responsive self-assembled DNA nanomachine that enables targeted ECL imaging of intracellular ATP in single cells [160]. The nanomachine features an ECL nanoemitter core constructed from Ru(bpy)₃²⁺-doped MOF (RuMOF), which encapsulates a high density of luminophores within its porous structure, and a DNA hydrogel shell containing ATP aptamer sequences that acts as a stimuli-gated barrier. The DNA gel shell physically hinders electron transfer between the TPrA co-reactant and the encapsulated luminophores, effectively quenching ECL emission. Upon specific binding of intracellular ATP to the aptamer sequences, the hydrogel shell dissociates, restoring ECL emission capability. The nanomachine achieved the first targeted ECL imaging of ATP distribution in single cells with high spatial resolution, revealing heterogeneous ATP distribution across different cell lines and subcellular regions through 3D ECL imaging at various heights from the electrode surface.

Lysate-based analysis relaxes the delivery constraint and thus allows fuller exploitation of the amplification and logic-processing capabilities of DNA nanostructures. Jia's group constructed a DTCHA system in which metastable hairpins were anchored on DTN vertices at a controlled ratio, generating hyperbranched three-dimensional DNA nanonetworks upon target initiation [72]. Owing to the spatial confinement effect, the local concentration of the hairpin probes was estimated to reach 147 μ M, so that the DTCHA reaction reached half-completion in 300 s (6.4-fold faster than conventional CHA), with an initial rate 12.4-fold higher. The resulting biosensor delivered a 2.9-fold stronger ECL signal and a detection limit of 0.63 fM for miR-21, nearly 52-fold lower than its CHA-based counterpart. By coupling this amplification module with YES and AND logic gates, the platform distinguished cancer cells from normal cells on the basis of miR-21 levels and further identified breast-cancer subtypes. Complementary to intracellular-marker analysis, cell-surface marker profiles can also be exploited for whole-cell phenotyping. Chen's group used a DNA-nanoribbon-templated copper nanocluster emitter combined with exonuclease III recycling to enumerate MUC1-overexpressing MCF-7 cells from 10 to 10⁵ cells/mL, with a detection limit of 5 cells/mL in diluted human serum [161]. This strategy illustrates how DNA-nanostructure-assisted ECL amplification can be extended from molecular biomarkers to intact-cell analysis.

Each mode has its own bottleneck. For *in situ* intracellular ECL detection, the main obstacles are poor cellular delivery, entrapment in endosomes, nuclease degradation, and the basic challenge of generating electrochemical excitation inside intact living cells. Lysate-based analysis avoids most of these problems, but it sacrifices spatial and temporal information. It can also be affected by marker degradation or uneven lysis during sample preparation. Connecting the two approaches remains a major challenge for cellular ECL bioanalysis. This could involve delivering DNA nanomachines that can amplify signals into living cells, or developing analytical strategies that keep spatial information intact during *ex vivo* analysis.

6. Conclusions and perspectives

In summary, DNA nanostructures form a flexible and programmable molecular toolbox that lets ECL biosensing move beyond the limits of conventional platforms. This review systematically examines structure-activity relationships across 1D, 2D, and 3D architectures. It shows that DNA nanostructures ease inherent drawbacks of ECL platforms through three complementary regulatory mechanisms. Ordered interfacial assembly improves electron-transfer efficiency and probe accessibility. Precise distance

modulation tunes resonance energy-transfer performance. Nanoconfinement effects accelerate local reaction kinetics. When these structural scaffolds are combined with established amplification strategies such as HCR, CHA, RCA, and DNA walker systems, and with emerging functional modules such as DNAzymes, molecular logic circuits, and CRISPR-Cas cascades, they enable ultrasensitive, highly specific, and smart-responsive detection for a broad range of analytes. Targets covered include pathogen nucleic acids, microRNAs, circulating tumor DNA, proteins, small molecules, and intracellular markers. These advances shift ECL biosensing from basic signal on/off transduction toward structurally sophisticated and functionally integrated bioanalytical systems.

Despite these advances, several systemic obstacles still hinder practical implementation. First are structural stability and batch-to-batch reproducibility. In complex biological fluids, DNA nanostructures are susceptible to nuclease degradation and conformational variations induced by ionic strength. Large-scale preparation also suffers from fabrication heterogeneity and weak sensing consistency. Second, *in vivo* compatibility and real-time monitoring remain underdeveloped. The long-term metabolic behavior of DNA nanostructures inside organisms has not been fully clarified. Most existing ECL setups depend on rigid electrochemical excitation, which is hard to realize in dynamic biological surroundings. Third, multiplexed detection is restricted by signal crosstalk originating from crowded surface probes, cross-reactivity among recognition elements, and non-specific adsorption within complex matrices such as serum and tissue fluid. Fourth, efforts toward ultra-low detection limits often sacrifice linear dynamic range. This persistent trade-off prevents simultaneous quantification of trace-level and high-abundance analytes in one single test. Fifth, clinical transformation is restrained by complicated operating procedures. Many amplification reactions demand strict thermal control and multi-step reagent tuning, which cannot match the requirements of portable and user-friendly devices. It should also be noted that higher amplification complexity does not necessarily mean better analytical performance. Multi-step cascades can improve sensitivity but often at the cost of increased background, longer assay time, and greater variability. For practical applications, a balanced combination of moderate amplification, low background, and high reproducibility is often more useful than an extremely low LOD achieved through a fragile multi-step workflow.

In order to break through the above development bottleneck, future research should focus on the deeper integration of ML and ECL technology. Although a small number of studies have focused on the integration of ML and ECL technologies, such as using deep learning to enhance ECL single-cell imaging [162] and assisting in the identification and characterization of individual bacteria [163], there is still a lack of systematic cross-fertilization studies. To improve structural stability, researchers may develop stimulus-responsive dynamic DNA nanostructures and adopt ML-assisted sequence optimization. Techniques such as generative adversarial networks and reinforcement learning can predict stable conformations and cut down experimental trial and error cycles. For *in vivo* and single-cell studies, near-infrared ECL luminophores, flexible or wireless excitation modes, and chemically modified DNA backbones with improved nuclease resistance are indispensable for real-time minimally-invasive monitoring. For multiplexed intelligent sensing, coupling DNA logic circuits with graph-neural-network-based multi-dimensional signal analysis helps build adaptive biomarker correlation models. This approach separates target signals from background noise and lowers false-positive rates for clinical specimens. To address the conflict between sensitivity and dynamic range, switchable amplification frameworks or ratiometric self-calibrating mechanisms should be explored. These designs can automatically adjust

signal gain according to target abundance. For clinical translation, microfluidic-integrated ECL chips and standardized ECL databases paired with patient-oriented calibration algorithms are required to narrow the gap between laboratory proof-of-concept research and point-of-care diagnosis. Joint advances along these routes will enable DNA-nanostructure-empowered ECL biosensing to achieve higher sensitivity, improved specificity and authentic intelligence. The technique will offer strong technical support for early-stage disease diagnosis, environmental monitoring and precision medicine.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. Specifically, the authors used DeepSeek, Kimi and ChatGPT for language polishing in entire manuscript. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Conceptualization, J.Z. and X.W.; writing—original draft preparation, J.Z. and X.W.; writing—review and editing, J.C.; supervision, J.C.; funding acquisition, J.C. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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