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REVIEW

Nanotechnology-Assisted Molecular Profiling: Emerging Advances in Circulating Tumor DNA Detection

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Abstract: Tumor-derived circulating cell-free DNA (ctDNA) has emerged as a pivotal biomarker for non-invasive cancer diagnosis, treatment monitoring, and prognostic evaluation. However, its inherently low abundance, high fragmentation, and rapid degradation impose stringent requirements on assay sensitivity, specificity, and analytical robustness. Rapid advances in nanotechnology have significantly accelerated progress in ctDNA detection. This review summarizes recent nanotechnology-assisted strategies for ctDNA analysis, including surface-engineered nanomaterials for selective enrichment, nano-enabled signal amplification modalities, and integrated platforms such as CRISPR-based detection, microfluidics and nanopore technologies. We further highlight nanostructure-based approaches for decoding methylation, fragmentation profiles, and multi-omics signatures, focusing on their potential to enhance early cancer detection and real-time therapeutic assessment. Moreover, increasing incorporation of artificial intelligence (AI) which spans nanostructure characterization, aptamer and probe design, multi-omics data integration, and algorithm development is reshaping the landscape of nano-assisted liquid biopsy. Finally, current challenges and future perspectives concerning the clinical translation of nanotechnology-assisted ctDNA detection are presented, emphasizing standardization, biocompatibility, automation, and regulatory readiness. Overall, this review provides a comprehensive outlook on how converging nanotechnology and AI innovations are advancing ctDNA-based precision oncology.

Keywords: nanomaterials, circulating tumor DNA, multi-omics, machine learning, cancer diagnostics

Introduction

Circulating tumor DNA (ctDNA), released into the bloodstream by apoptotic and necrotic tumor cells, has emerged as a promising biomarker for non-invasive liquid biopsy.[1] Compared with conventional tissue biopsies, ctDNA analysis offers significant advantages, including minimal invasiveness, dynamic monitoring of tumor evolution, and the potential to guide personalized therapy.[2] Conventionally, the primary methodologies for ctDNA analysis can be broadly divided into PCR-based methods and sequencing-based strategies. However, the clinical translation of ctDNA detection still faces critical challenges.[3] The concentration of ctDNA in plasma is extremely low and often obscured by abundant background cell-free DNA (cfDNA) derived from normal cells. Furthermore, ctDNA exhibits high heterogeneity in length, methylation patterns, mutation spectra, and high fragmentation, making its accurate detection more challenging.[4]

Nanotechnology provides unique opportunities to overcome these barriers.[5] Owing to their high surface-to-volume ratio, versatile surface chemistry, and tunable physicochemical properties, nanomaterials have been widely utilized to enhance ctDNA enrichment, recognition, and signal amplification.[6] The continual optimization and surface functionalization of nanomaterials have led to the development of more efficient and specific platforms for liquid biopsy applications. Chemical modifications of nanomaterials, such as the conjugation of antibodies or aptamers, significantly

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enhance the capture efficiency and reduce background noise, making ctDNA detection more accurate and reliable. These innovations are critical for advancing the clinical applicability of ctDNA-based liquid biopsy, particularly for personalized medicine. Recent advances have demonstrated that nanotechnology can be integrated with molecular tools such as clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems, microfluidics and nanopore sequencing.[7–9] These interdisciplinary approaches not only improve the sensitivity and specificity of ctDNA detection but also enable novel applications in epigenetic profiling and multi-omics integration for early cancer diagnosis.[1,10,11]

In recent years, the rapid advancement of artificial intelligence (AI) and machine learning (ML) has profoundly reshaped the field of biomedical nanotechnology. Cutting-edge deep learning frameworks, generative AI models, and data-driven molecular design algorithms have enabled the rational engineering of nucleic acid aptamers, nanoprobes, and surface-functionalized nanomaterials with unprecedented precision and efficiency. These computational strategies facilitate the accurate prediction of binding affinities, conformational dynamics, and sequence–structure–function relationships, thereby accelerating the discovery of high-performance aptamers and nanoscale biosensors for ctDNA recognition. Furthermore, AI-empowered analytical pipelines have become integral to the processing of ctDNA and multi-omics datasets, enhancing signal deconvolution, variant detection, methylation landscape reconstruction, and fragmentomic pattern interpretation with superior sensitivity, reproducibility, and scalability.[12]

Nevertheless, despite these advances, existing reviews often focus either on nanomaterial-based biosensing or ctDNA detection technologies in general. A review that delivers mechanistic insights linking nanotechnology innovations with the emerging fields of fragmentomics, epigenomics, and multi-omics analysis is currently lacking but highly needed.[13] This disconnected gap limits our understanding of how nanotechnology drives breakthroughs in ctDNA-based liquid biopsy.[14]

This review provides a comprehensive overview of recent advancements in five years in nanotechnology-enabled ctDNA detection strategies, with a focus on the entire analytical workflow, from nucleic acid capture, molecular enrichment, and signal amplification to single molecule reading and multi-dimensional molecular interpretation. We summarize the latest developments in how nanotechnology enhances ctDNA detection and its integration with cutting-edge molecular tools such as CRISPR and microfluidics. Furthermore, we highlight how nanotechnology is facilitating studies in fragmentomics, epigenetic profiling, and multi-omics integration, opening up new opportunities for cancer diagnosis and treatment monitoring. The integrated landscape of these nanotechnology-assisted ctDNA analysis is illustrated in Figures 1 and 2. Finally, we discuss the translational challenges facing the clinical application of these technologies and explore potential future directions for improving the sensitivity, specificity, and clinical applicability of ctDNA diagnostics.

Together, this review forms a cohesive framework that integrates nanomaterials science, molecular diagnostics, data-driven analytics, and translational applications.

Upstream Workflow: ctDNA Capture and Enrichment Strategies

The analytical performance of ctDNA assays is fundamentally constrained by the efficiency with which rare tumor-derived fragments can be isolated from a vast background of cfDNA. The biological characteristics of ctDNA place exceptional demands on the upstream processing steps. Recent advances in nanotechnology have introduced highly tunable interfaces and engineered nanoscale environments capable of enhancing molecular selectivity and facilitating rapid separation. Through precise control of surface chemistry, physicochemical interactions, and nanoengineered platforms enable the preferential capture and enrichment of ctDNA. These developments establish the foundation upon which sensitive signal transduction, multidimensional profiling, and ultimately reliable clinical interpretation can be achieved.

Surface Functionalization Strategies

The Concept of Surface Functionalization

Surface functionalization of nanomaterials refers to the modification of their surfaces to enhance their interaction with specific targets, which is crucial for applications such as ctDNA detection. Historically, surface functionalization has evolved from simple physical adsorption to precise molecular engineering. While early strategies relied on passive non-

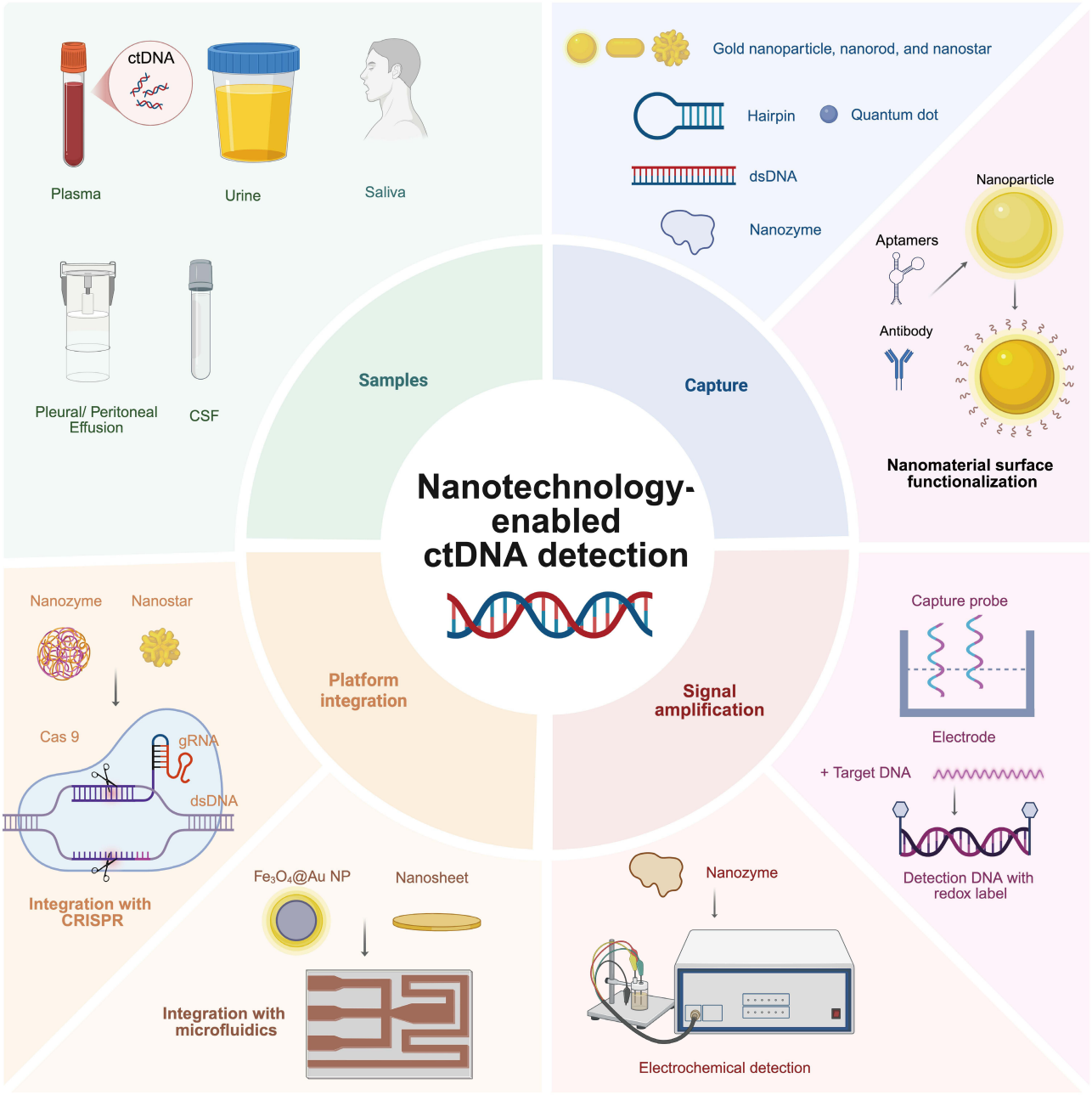

Figure 1 Overview of nanotechnology-enabled strategies for ctDNA detection. The schematic illustrates how nanotechnology addresses the challenges of low ctDNA abundance and complexity through four key technological modules. Samples: Diverse biofluids serve as non-invasive sources for liquid biopsy. Capture: To overcome low target concentration, nanomaterial surface functionalization utilizes high-surface-area scaffolds (gold nanoparticles, nanorods, nanostars, and quantum dots) conjugated with high-affinity recognition elements to specifically enrich trace tumor-derived fragments. Signal amplification: Innovative transduction strategies, such as nanozyme-mediated catalysis and electrochemical redox cycling, convert subtle molecular binding events into robust, quantifiable signals, significantly enhancing detection sensitivity. Platform integration: The convergence of nanostructures with CRISPR/Cas systems for single-nucleotide resolution and microfluidics for rapid, automated processing represents a technological breakthrough in achieving high-specificity diagnostics. Arrows indicate the sequential workflow from sampling to analysis and the direction of molecular interactions; bold text denotes the four primary functional stages of the detection process.

covalent interactions often limited by poor stability, the field advanced to robust covalent coupling methods such asthiol-gold chemistry. Currently, the focus has shifted toward site-specific and spatially controlled engineering utilizing tools like DNA nanostructures to maximize target accessibility and recognition efficiency. This functionalization allows for the specific capture and enrichment of ctDNA, improves stability in complex biological environments, builds signal amplification systems, enables multifunctional integration, and enhances biocompatibility. Optical nanomaterials with

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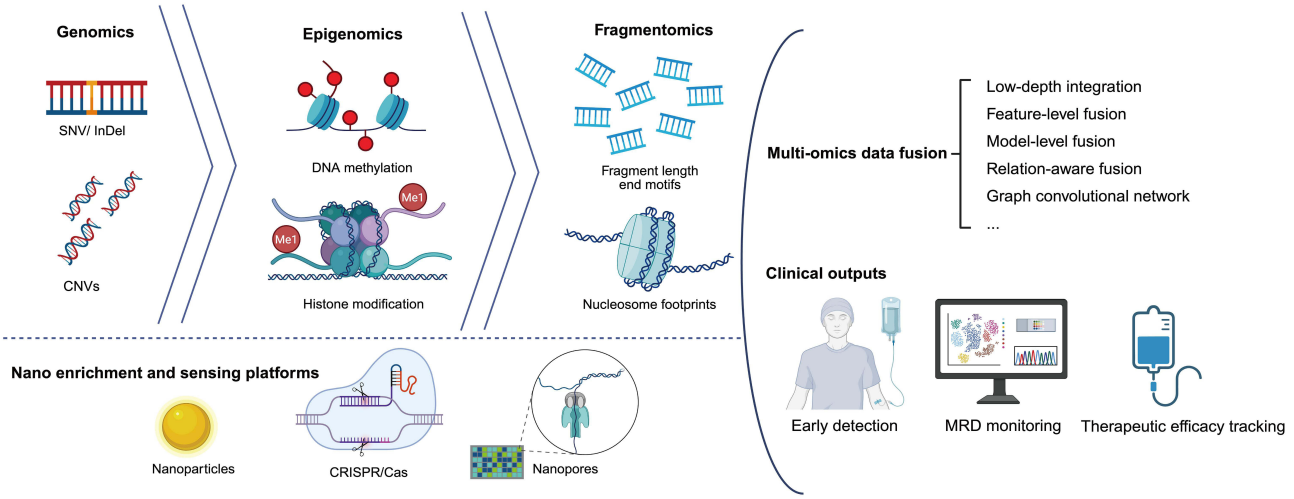

Figure 2 Integration of nanotechnology, multi-omics detection and machine learning. The diagram illustrates a holistic workflow for precision liquid biopsy. Molecular Dimensions: The upper panel depicts the comprehensive characterization of ctDNA across Genomics (SNVs/InDels, CNVs), Epigenomics (DNA methylation, histone modifications), and Fragmentomics (fragment length, end motifs, nucleosome footprints). Technological Enablers: Nano enrichment and sensing platforms, including functionalized nanoparticles, CRISPR/Cas biosensors, and nanopore sequencing, serve as the high-sensitivity hardware interface to capture and digitize these heterogeneous molecular features. Data Fusion: A sophisticated Multi-omics data fusion module utilizes advanced machine learning architectures to synthesize diverse data streams. This computational integration overcomes the limitations of single-omics assays by maximizing information extraction from low-abundance signals. Clinical Utility: The processed output translates into actionable Clinical outputs, enabling precise early detection, MRD monitoring, and longitudinal therapeutic efficacy tracking.

antibody or aptamer-based surface functionalization have shown great promise in ctDNA detection, enabling highly specific recognition of tumor-derived sequences amidst abundant background DNA. The nanostructure surface engineering for ctDNA biosensing is illustrated in Figure 3.

Antibody-Based Surface Functionalization

Antibody-mediated surface modification exploits the exquisite antigen-antibody recognition mechanism to achieve molecular specificity in ctDNA detection platforms. Gold nanoparticles (AuNPs) have emerged as particularly attractive scaffolds due to their biocompatibility and tunable surface chemistry. Through thiol-gold chemisorption or carbodiimide-mediated crosslinking, antibodies targeting methylated DNA-binding proteins or mutation-specific epitopes can be densely immobilized onto AuNP surfaces, creating high-avidity capture interfaces.[6] When integrated with LSPR or SERS transduction, these antibody-functionalized AuNPs enable real-time monitoring of ctDNA binding events with attomolar-level detection limits.[15]

The spatial organization of antibodies on nanomaterial surfaces critically influences assay performance. Controlled antibody orientation achieved through site-specific conjugation via Fc-binding proteins or engineered cysteine residues ensures that antigen-binding fragments remain accessible for target engagement, thereby enhancing binding kinetics and reducing nonspecific adsorption compared to random attachment strategies.[15] Nanoscale surface curvature can further promote multivalent antibody-antigen interactions, effectively increasing functional avidity beyond monovalent binding. Despite these advantages, antibody-based functionalization faces inherent limitations including batch-to-batch variability, susceptibility to proteolytic degradation, and restricted operating conditions. The relatively large molecular size of antibodies (~150 kDa) also constrains surface loading density and may introduce steric hindrance in densely packed sensor architectures, motivating the exploration of alternative molecular probes such as aptamers.

Advantages of Aptamers

Aptamers, single-stranded DNA or RNA oligonucleotides typically ranging from 20 to 100 nucleotides, offer compelling advantages over antibodies as molecular recognition elements in nanotechnology-based ctDNA detection. Selected through systematic evolution of ligands by exponential enrichment (SELEX), aptamers fold into distinct three-dimensional conformations that enable high-affinity binding to diverse targets including nucleic acids, proteins, small molecules, and even whole cells.[18] Unlike antibodies, aptamers can be chemically synthesized with high reproducibility

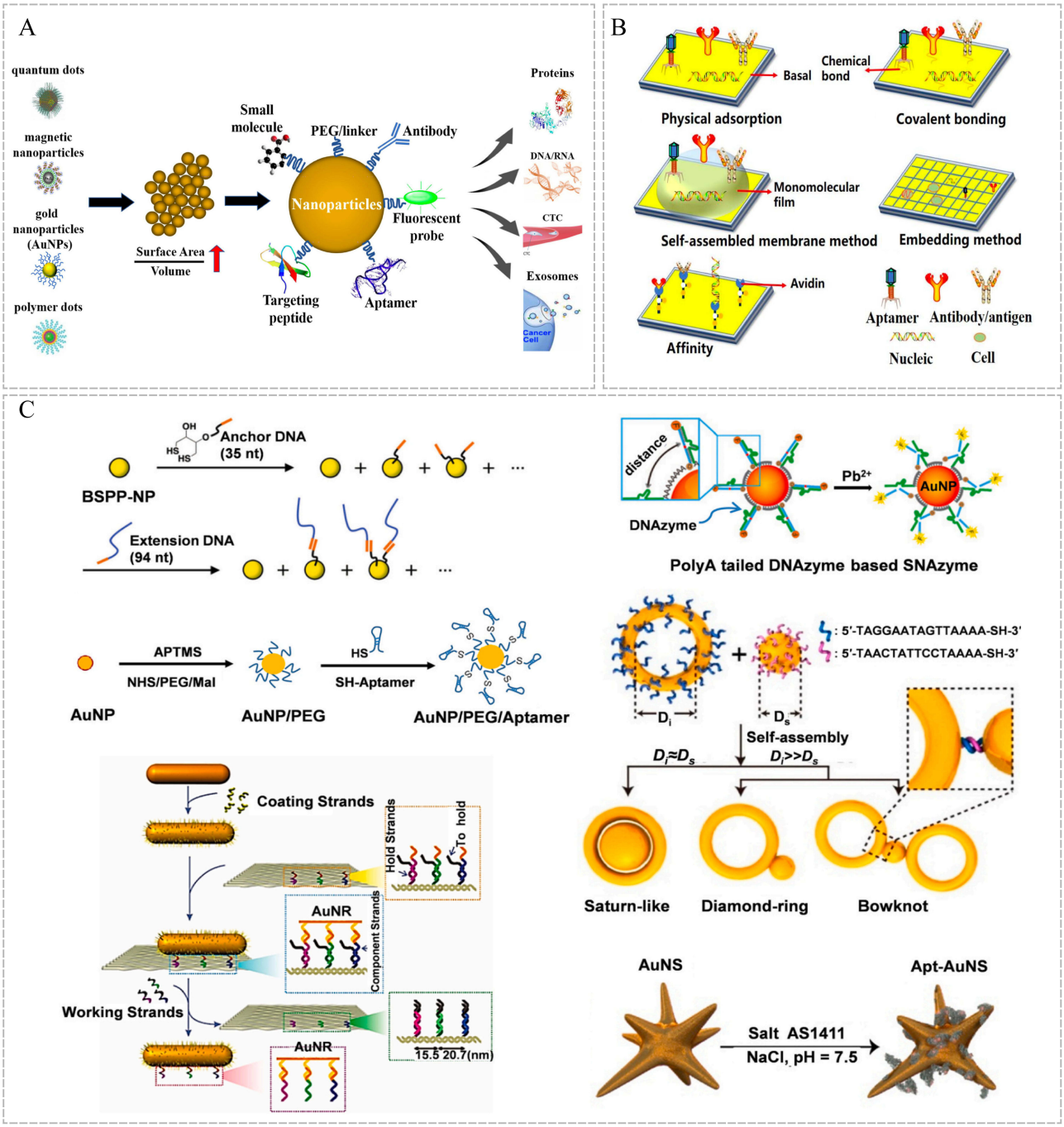

Figure 3 Nanostructure surface engineering for ctDNA biosensing. (**A**) High–surface-area nanomaterials such as Au nanoparticles/rods/stars, magnetic nanoparticles, polymer dots, and quantum dot provide versatile platforms that can be densely decorated with capture and reporting elements, such as antibodies, small molecules, peptides, fluorophores, and nucleic-acid aptamers, enabling selective recognition of cancer-related targets. Reproduced with permission from reference.[15] Copyright the Author(s) 2019. (**B**) Common strategies for fixing biomolecular receptors to transducer surfaces include physical adsorption, covalent coupling, self-assembled monolayers, matrix embedding, and affinity-based attachment such as biotin–avidin. Reproduced with permission from reference.[16] Copyright 2022 Biosensors. (**C**) DNA engineering on gold nanomaterials including thiolated anchor strands, PEG-mediated coupling, DNA origami patterning, and poly(A)-based SNAzyme assembly which supports programmable and versatile nanostructures for biosensing applications. Reproduced with permission from reference.[17] Copyright 2023 Elsevier.

and minimal batch-to-batch variation, eliminating the need for animal immunization and ensuring consistent performance across production cycles.[19] Their smaller molecular size, typically 5–15 kDa while ~150 kDa for IgG antibodies, permits higher surface loading density on nanomaterial substrates, reduces steric hindrance, and facilitates deeper tissue penetration in potential in vivo applications.[20] Aptamers also exhibit remarkable thermal stability, retaining functionality

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after repeated denaturation-renaturation cycles that would irreversibly denature antibodies, and they tolerate a broader range of pH, ionic strength, and organic solvents encountered in clinical sample processing.[19]

The chemical versatility of aptamers enables straightforward site-specific modification with functional groups for oriented surface conjugation. Thiol, amine, or biotin moieties can be introduced at precise positions during oligonucleotide synthesis, allowing controlled attachment to gold nanoparticles, magnetic beads, or carbon nanomaterials through well-established bioconjugation chemistries.[21] Furthermore, aptamers can be rationally engineered to incorporate stimuli-responsive elements such as pH-sensitive i-motif structures or light-activatable azobenzene modifications, enabling triggered release of captured ctDNA for downstream analysis or dynamic control of sensor activation states. Their nucleic acid backbone also facilitates seamless integration with DNA nanotechnology platforms including DNA origami, tetrahedral nanostructures, and hybridization chain reaction (HCR) circuits, creating sophisticated signal amplification architectures unattainable with protein-based probes.[17] Perhaps most critically for ctDNA applications, aptamers can be selected to discriminate single-nucleotide variants with extraordinary precision, recognizing mutation-specific secondary structures or altered base-pairing patterns that distinguish oncogenic alleles from wild-type sequences, which is a level of specificity challenging to achieve with conventional antibodies.

Applications of Aptamers in ctDNA Detection

The integration of aptamers with nanotechnology platforms has enabled sensitive and specific ctDNA detection through diverse mechanisms. In optical biosensing, aptamer-functionalized gold and silver nanoparticles have been incorporated into SERS-based microfluidic chips for ctDNA analysis. Park et al engineered dispersible Fe_3O_4-Au core-shell magnetic nano-electrodes with aptamer functionalization, achieving ultrasensitive ctDNA detection with a limit of detection down to 3 aM (approximately 2 copies per microliter) through repeated electrochemical measurements and progressive signal amplification.[6] Advanced architecture can combine aptamers with DNA nanotechnology for cascade amplification. DNA tetrahedral structures and other nano frames incorporating aptamer sequences provide rigid three-dimensional scaffolds that orient recognition elements optimally while minimizing nonspecific adsorption.[17,21] When integrated with catalytic hairpin assembly (CHA) or hybridization chain reaction (HCR), these platforms initiate autonomous polymerization upon aptamer-target binding, generating amplified signals. The nucleic acid nature of aptamers facilitates integration with CRISPR/Cas systems. Aptamer pre-concentration of low-abundance ctDNA onto magnetic microbeads locally elevates target concentration, enhancing subsequent Cas12a or Cas13a trans-cleavage efficiency for multiplex bioassays.[7,22] In electrochemical platforms, aptamer conjugation to carbon nanotubes or graphene oxide leverages high electrical conductivity to transduce binding events into measurable current changes, with the smaller aptamer size permitting higher surface loading density on nanoelectrodes.[5,17]

Microfluidic Platforms for Low Volume and High Selectivity Enrichment

Nanotechnology fundamentally enhances the performance of ctDNA detection workflows, especially when integrated into microfluidic platforms, by overcoming the diffusion limitations inherent in bulk fluids. Nanomaterials are central to advanced microfluidic technology, leveraging their high surface-to-volume ratios and tunable surface chemistries to significantly enhance molecular recognition and enrichment efficiency. Studies show that a no-pump SERS microfluidic chip engineered with AuNPs and AgNPs achieves efficient ctDNA capture and completes the whole analysis process within 5 minutes, significantly improving sensitivity.[9] This platform exploits the localized electromagnetic field enhancement generated by plasmonic nanomaterials to provide orders of magnitude higher detection sensitivity than traditional methods, while avoiding the need for external equipment. Further research indicates that coupling the unique photothermal and interfacial properties of nanomaterials with isothermal amplification technology, such as recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP), can significantly accelerate reaction kinetics and enhance ctDNA enrichment through localized heat generation and high-density molecular loading. By embedding nanoparticles into microfluidic architectures to create highly reactive solid-phase interfaces, researchers have developed a novel nanoplatform, greatly increasing detection sensitivity by maximizing target capture within picoliter-scale reaction volumes.[23] Additionally, employing nanoporous membranes or nanoparticles leverages nanoscale size-exclusion principles and differential adsorption affinities to enable the selective separation of short-fragment ctDNA from long-fragment genomic DNA.[24] Representative microfluidic and nanostructure-assisted platforms are illustrated in Figure 4

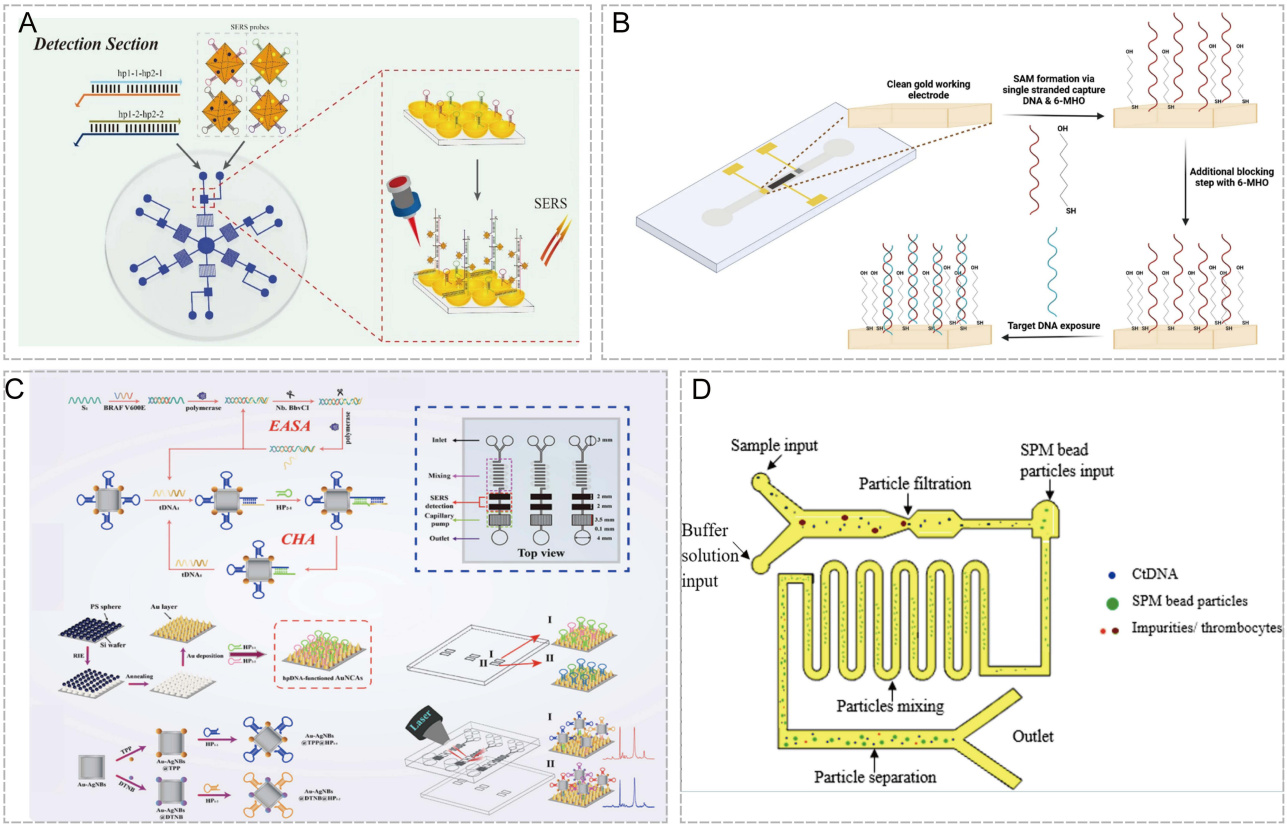

Figure 4 Representative microfluidic and nanostructure-assisted platforms for ctDNA detection. (**A**) This platform eliminates the need for bulky external pumps by leveraging capillary forces for fluid transport. It integrates gold nanostructured probes with a cascade signal amplification strategy, achieving a technological breakthrough in portability while maintaining ultrasensitive detection for gastric cancer biomarkers. Reproduced with permission from reference.[25] Copyright The Author(s) 2022. (**B**) A nanointerface-on-chip is constructed on MEMS-fabricated thin-film Au/Pt/Ag electrodes. The innovation lies in the precise Self-Assembled Monolayer (SAM) engineering, which enables the pre-enrichment-free and selective detection of ctDNA directly from complex fluids, drastically reducing turnaround time. Reproduced with permission from reference.[26] Copyright The Author(s) 2024. (**C**) This advanced platform combines enzyme-assisted signal amplification (EASA) and catalytic hairpin assembly (CHA) with a Gold Nanocone Array (AuNCA) substrate. The specific nanocone geometry generates high-density electromagnetic hotspots, significantly enhancing Raman scattering signals for early lung cancer prognosis. Reproduced with permission from reference.[27] Copyright 2023 Qian et al (**D**) A microfluidic separation system utilizes superparamagnetic (SPM) beads to isolate ctDNA from whole blood. The serpentine channel design maximizes particle–target mixing, while the magnetic field facilitates the rapid removal of blood cells and impurities, solving the bottleneck of sample purity in liquid biopsy. Reproduced with permission from reference.[28] Copyright The Author(s) 2021.

Midstream Workflow: Nanotechnology-Enhanced Signal Amplification

Following the selective capture and enrichment of ctDNA, the next critical challenge lies in converting these scarce molecular targets into detectable signals with sufficient magnitude and specificity. Nanotechnology offers a diverse repertoire of optical, electrochemical, catalytic, and CRISPR-coupled strategies that enhance the signal transduction. By leveraging localized electromagnetic field confinement, accelerated electron transfer, enzyme-mimetic catalysis, or targeted nucleic acid cleavage, nanoengineered amplification platforms can transform minute molecular recognition into robust, quantifiable outputs. These advances not only push the detection limits toward the single-molecule regime but also enable rapid and multiplexed analysis compatible with clinical workflows.

Optical Nanomaterials for Signal Amplification

The concentration of ctDNA in blood is extremely low and often masked by abundant normal cfDNA, thereby necessitating highly sensitive signal amplification technologies for accurate detection. A variety of signal readout methods are employed in ctDNA detection, such as colorimetric changes, fluorescence emission, electrochemical signals, and techniques like surface plasmon resonance (SPR) or Raman spectroscopy.[5] Fluorescence-based sensors represent a crucial branch of nanotechnology applications for ctDNA detection. For instance, a label-free fluorescent sensor utilizing $CsPbBr_3$ nanosheets was developed, where fluorescence quenching by Ti_3C_2Tx enables sensitive ctDNA detection through intensity changes

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before and after target hybridization.[29] Additionally, an AIE based fluorescence probe (TPE-DNA) was constructed using a copper-free click reaction for sensitive detection of ctDNA. The fluorescence is turned on upon target-induced probe aggregation.[30] Other innovative strategy involves an HCR-FRET fluorescent sensing system.[31] A homogeneous, extraction-free dual-signal sensing platform is constructed by integrating rolling circle amplification (RCA)-based DNA hydrogels with DNA@Cu^{2+} nanospheres for the simultaneous detection of PIK3CA and ESR1 mutations. In this system, target binding triggers hydrogel disassembly to expose G-quadruplex sequences for ThT fluorescence enhancement ("signal-on"), while concomitantly releasing Cu^{2+} ions to quench quantum dot emission ("signal-off"). This synergistic approach achieves attomolar-level sensitivity within 40 minutes, offering a rapid diagnostic tool for breast cancer that bypasses complex nucleic acid extraction steps.[32]

Electrochemical Nanointerfaces for Amplified Detection

Recent advances in electrochemical biosensors have significantly enhanced the efficiency of ctDNA analysis, paving the way for automated liquid biopsy and point-of-care testing. These biosensors leverage the unique properties of nanostructures, which can be used to construct DNA circuits for signal amplification and highly sensitive detection. By employing dispersible Fe_3O_4-Au core-shell magnetic nano-electrodes with repeated electrochemical measurements, ctDNA signals can be progressively amplified, enabling high detection sensitivity down to 3 aM and allowing for rapid ctDNA analysis.[6] Additionally, ZnIn2S4@AuNPs composite electrode facilitate ctDNA identification by monitoring electron transfer changes during the photoelectrochemical process. This method enables highly sensitive signal-on electrochemical detection.[33] Furthermore, nanostructures such as DNA origami, tweezers, rollers, and walkers are being integrated into DNA circuits, enhancing the specificity and efficiency of ctDNA detection through precise molecular recognition and signal amplification.[5] The integration of multimodal signals holds great potential in ctDNA detection. Multimodal detection integrating electrochemical and fluorescence signals shows significant advantages in ctDNA analysis. The dual-signal system enhances sensitivity, expands the linear detection range, and improves anti-interference capability through mutual signal validation. Fluorescence provides primary target recognition, while the electrochemical output, amplified via nanomaterial-based coupling, enables reliable detection of low-abundance EFGR mutations.[34]

Nanozyme-Based Catalytic Amplification

In recent years, nanozymes, which are the nanomaterials with enzyme-mimicking catalytic activity, have been extensively integrated into biosensor systems.[35] Co_3O_4 nanozyme played a pivotal role in a photoelectrochemical (PEC) biosensing strategy for ultrasensitive detection of ctDNA, where it catalyzed the precipitation reaction that triggered triple quenching of the PEC signal from $MgIn_2S_4$. In the presence of H_2O_2, the nanozyme facilitated the formation of an insulating red precipitate, effectively turning off the PEC signal.[36] Fe single-atom carbon dot nanozymes (SA Fe-CDs) catalyzed the oxidation of tetramethylbenzidine (TMB), effectively amplifying the electrochemical signal with excellent catalytic activity, thereby enhancing ctDNA detection sensitivity.[37] CoB NS nanozyme enabled ctDNA detection via dual-mode signal transduction. Its peroxidase-like and catalase-like activities produced color and oxygen, supporting both colorimetric and pressure-based readouts.[38] The oxidase-mimicking MnB_2 nanozyme was employed in a sandwich-type sensing strategy, where tis catalytic properties enabled effective colorimetric signal generation.[39] Representative nanotechnology-enabled signal amplification strategies are summarized in Figure 5. Nanozymes with distinct catalytic activities have been applied across various ctDNA detection strategies, contributing to signal generation through oxidation, precipitation and many other amplification mechanisms. A comparison of signal amplification methods enhanced by nanotechnology for ctDNA detection is summarized in Table 1.

CRISPR–Nanomaterial Hybrid Amplification

Although the conventional detection of ctDNA relies heavily on amplification techniques like PCR due to its low abundance in blood, these methods are hampered by inherent limitations, including the potential for introduced sequence artifacts and prolonged turnaround times.[5] Historically, CRISPR diagnostics have evolved from simple enzymatic assays often requiring pre-amplification to sophisticated nanostructured systems. Initial integration with nanomaterials such as AuNPs enhanced portability via visual readouts, while current advancements leverage complex interfaces such as metal-

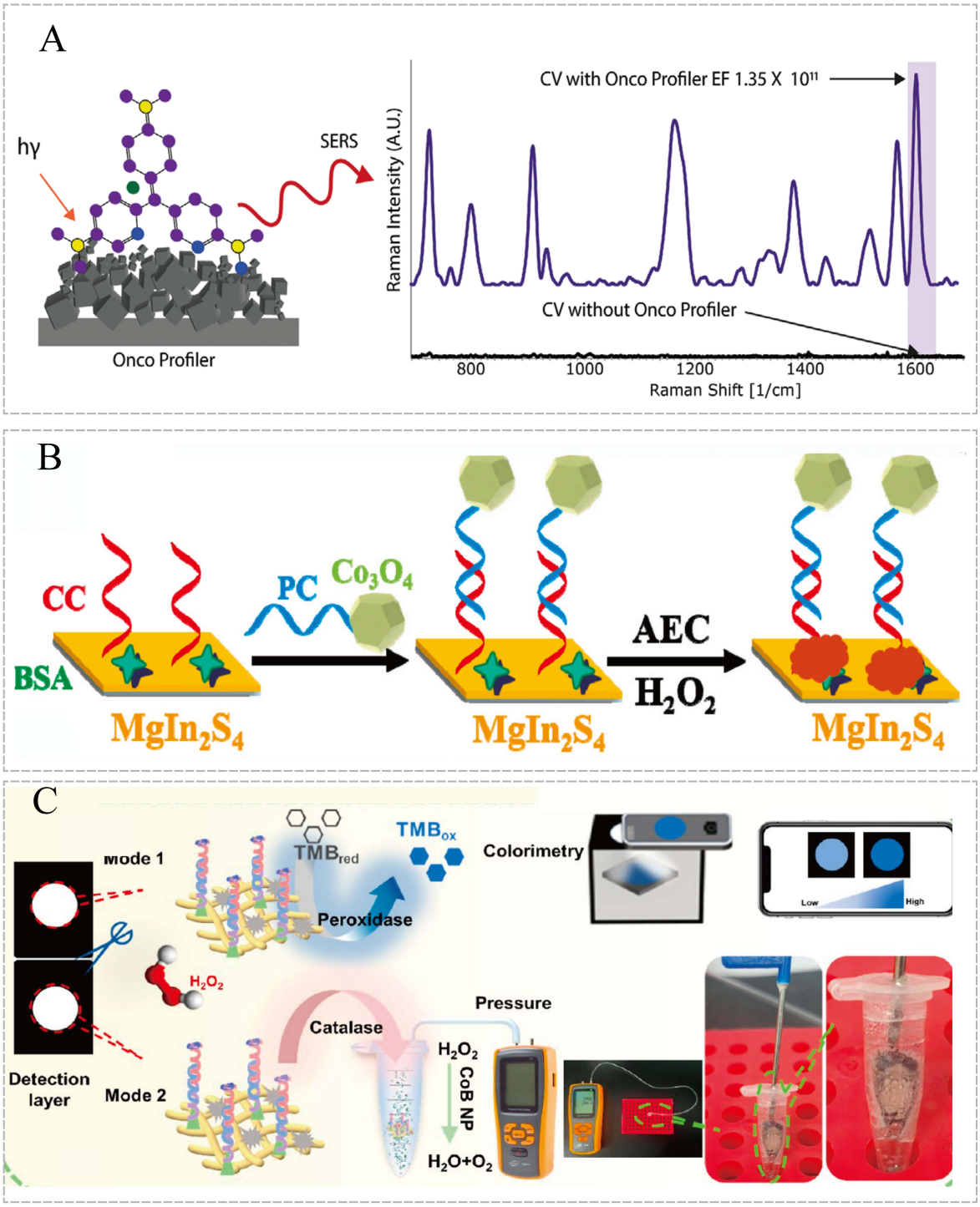

Figure 5 Representative nanotechnology-enabled signal amplification strategies for ctDNA detection. (**A**) Schematic shows DNA molecules adsorbed on nanostructured plasmonic surfaces generating intensified Raman signals upon laser excitation (hγ). Comparative spectra demonstrate substantial enhancement with OncoProfiler (upper, CV = 1.35×10^{11}) versus unmodified substrates. Distinct spectral fingerprints at 800–1600 cm^{-1} enable differentiation of breast, colorectal, and lung tumor DNAs. This electromagnetic field enhancement achieves single-molecule sensitivity and cancer-specific profiling without complex preprocessing, representing a breakthrough in label-free optical detection Reproduced with permission from reference.[40] Copyright 2022 Wiley-VCH GmbH. (**B**) Co_3O_4 nanozyme-catalyzed precipitation coupled with enzyme-free amplification enables ultrasensitive ctDNA detection on $MgIn_2S_4$ photoelectrodes. Sequential mechanism is to target capture and probe circularization, Co_3O_4 recruitment and H_2O_2-mediated precipitation via AEC coupling, generating insulating precipitate that quenches signal through physical barrier formation, competitive light absorption, and charge recombination. This innovative triple-quenching mechanism achieves femtomolar detection limits with exceptional signal-to-background ratio. Reproduced with permission from reference.[36] Copyright 2024 Elsevier. (**C**) Bifunctional CoB nanosheets exhibit peroxidase-like and catalase-like activities enabling dual readout: Mode 1 - peroxidase activity catalyzes TMB oxidation for colorimetric detection; Mode 2 - catalase activity generates O_2 for pressure-based quantification using smartphone sensors. Right panels show colorimetric gradient, portable pressure measurement, and pop-up paper structure for automated reagent mixing. Platform achieves attomolar sensitivity with low cos, rapid results less than 30 minutes and equipment-free operation, bridging laboratory performance with point-of-care accessibility for decentralized cancer screening. Reproduced with permission from reference.[38] Copyright 2024 American Chemical Society.

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Table 1 Representative Nano-Enabled Signal Amplification Techniques for ctDNA Detection

Signal Amplification Technique	Principle	Advantages	Limitations	Application Examples	References
Surface Enhancement of Raman Scattering (SERS)	Enhances Raman scattering signals using nanomaterials for highly sensitive detection.	Extremely high sensitivity, capable of single-molecule detection, suitable for low-abundance ctDNA detection.	Requires complex equipment and sample preparation, sensitive to sample matrix interference.	Used for ctDNA detection, combined with gold nanocone array and SERS technology for early lung cancer detection.	[27,40]
Fluorescence Resonance Energy Transfer (FRET)	Detection of DNA binding via energy transfer between two fluorescence probes, where nanoparticles such as quantum dots or gold nanoparticles act as mediators to amplify fluorescence signals upon ctDNA hybridization.	High sensitivity and multiplexing capability enabled by nanoscale distance control and enhanced resonance energy transfer efficiency.	Requires high-quality probes, susceptible to environmental and fluorescence interference.	Applied in an excitation/emission-enhanced heterostructure photonic crystal array coupled with a "DD-A" FRET entropy-driven circuit for ultrasensitive and high-resolution ctDNA detection (LOD: 12.9 fM; 0.01% VAF discrimination)	[41]
Colorimetric Reaction	Nanomaterial-mediated colorimetric detection based on localized surface plasmon resonance (LSPR) of gold nanoparticles or catalytic oxidation by nanozymes, where ctDNA hybridization or target recognition induces visible color change for rapid on-site analysis.	Simple operation, low cost, and fast, suitable for resource-limited settings.	Lower sensitivity, prone to background interference.	Used in low-cost portable devices for ctDNA detection in colon carcinoma	[42]
Electrochemical Signal Amplification	Detects ctDNA through electrode and current changes, signal enhanced by nanomaterials.	High sensitivity, suitable for high-throughput detection and rapid screening.	Electrodes prone to contamination, higher cost for devices and reagents.	Used in ctDNA electrochemical detection with SA Fe-CDs nanozymes. A detection sensitivity down to 1.26 aM was achieved with a signal-to-noise ratio of 3.	[37]
Nanozyme Reaction	Uses nanomaterials with enzyme-like catalytic activity to amplify the signal, often combined with electrochemical or colorimetric methods.	Multifunctional, capable of amplifying signals and recognizing targets simultaneously, suitable for rapid detection.	Stability and reactivity of nanozymes can be affected by the environment, catalytic efficiency related to surface properties of materials.	Used in electrochemical or colorimetric detection platforms to enhance sensitivity for detecting cancer-related gene mutations, such as BRAFV600E, KITL576P, and NRASQ61K in melanoma ctDNA, achieving 0.005% variant detection sensitivity directly from unprocessed plasma.	[36,37,43]

(*Continued*)

Table 1 (Continued).

Signal Amplification Technique	Principle	Advantages	Limitations	Application Examples	References
Isothermal Amplification	Nanomaterials enhance isothermal amplification by providing localized heating, template enrichment, and nanointerface–mediated signal transduction, enabling high-efficiency ctDNA detection under constant temperature.	Does not require high-temperature cycling, simple operation, usable in environments with poor temperature control.	Requires high control over the amplification process, susceptible to false positives.	Applied in CRISPR/Cas12a-integrated LAMP platforms for detecting KRAS mutations in plasma ctDNA. Achieves ultra-high sensitivity as 0.1% VAF, approximating femtomolar-level detection limits for early screening and therapy monitoring	[44–48]
Polymer Chemistry Amplification	Uses polymers to enhance ctDNA signal through specific polymer-catalyzed reactions. Nanostructures act as catalytic scaffolds and signal mediators in polymerization-based amplification, enhancing ctDNA signal output through localized polymer growth and nanointerface-driven electron transfer.	Suitable for high-sensitivity detection, reduces background interference, enhances accuracy of signal detection.	Stability and reaction time of polymers need strict control, material selection is complex.	Employed in polymerization chain reaction–based biosensors, such as rolling circle amplification (RCA) coupled with polymer-catalyzed signal enhancement	[44,49]

organic frameworks to achieve amplification-free, ultrasensitive detection suitable for clinical use. In recent years, CRISPR – Cas gene-editing system, owing to its highly precise nucleic acid recognition capability, has shown great potential in ctDNA detection, particularly when integrated with nanotechnology.[22] The CRISPR-Cas systems (eg Cas 9, Cas12a, Cas13) activate collateral cleavage activity after recognizing specific DNA or RNA sequences, thereby degrading surrounding fluorescent reporter molecules and generating a detectable signal. This property not only eliminates the need for PCR pre-amplification and simplifies the detection workflow, but also enables accurate discrimination of single-nucleotide variants (SNVs), making CRISPR–Cas a powerful tool for highly sensitive and specific nucleic acid detection in liquid biopsy applications.[50]

The synergy between the CRISPR/Cas system and nanotechnology has enabled the development of diverse and efficient ctDNA detection strategies.[51] The CRISPR/Cas12a system targets the EGFR L858R mutation, with two Cas12a proteins independently regulating fluorescence and electrochemical signals for cascade amplification. By integrating nanomaterials into this system, such as AuNPs or carbon nanotubes (CNTs), the electrochemical signals can be accurately quantified. This approach enables precise identification of low-abundance mutations.[34] Wu et al used the CRISPR/Cas12a system, combined with manganese metal-organic frameworks (Mn-MOFs), enables highly sensitive detection of ctDNA and identification of single nucleotide variants without pre-amplification.[52] The integration of nanomaterials in the CRISPR/Cas system not only enhances signal amplification but also facilitates more accurate and reliable quantification of specific mutations, such as EGFR L858R, in complex biological samples. To further mitigate off-target effects, a novel AND logic-gated DNA nanodevice was engineered by coupling the CRISPR/Cas9 nickase (nCas9) system with hybridization chain reaction (HCR). In this topology, signal amplification is strictly conditional upon a dual-recognition event which is nCas9-mediated strand nicking and specific hairpin probe invasion, thereby

ensuring that signals are generated only when the exact target sequence is present. This logic-driven strategy effectively eliminates false positives and achieves femtomolar-level sensitivity (LOD ~1 fM) for discriminating single-nucleotide variants such as KRAS G12D and EGFR T790M in complex serum matrices.[53]

The integration of nanotechnology with CRISPR has fundamentally reshaped the paradigm of ctDNA detection. CRISPR systems (eg, Cas9, dCas9, Cas12a) provide unparalleled molecular recognition precision, enabling specific cleavage or binding of target sequence.[54] Meanwhile, nanotechnology empowers detection platforms through signal amplification (eg, AuNP-based cascade amplification),[7,55,56] multiplex detection via nanoscale probes carrying multiple targets.[22] Furthermore, nanomaterials facilitate amplification-free detection with detection limits (LOD) down to the femtomolar range.[57] Together, these advances overcome the major clinical barriers of ctDNA detection: low abundance, high background noise, and short half-life, and drive liquid biopsy towards portability, ultra-sensitivity, and high specificity.[58] Figure 6 illustrates typical strategies that couple CRISPR/Cas molecular recognition with nanotechnology.

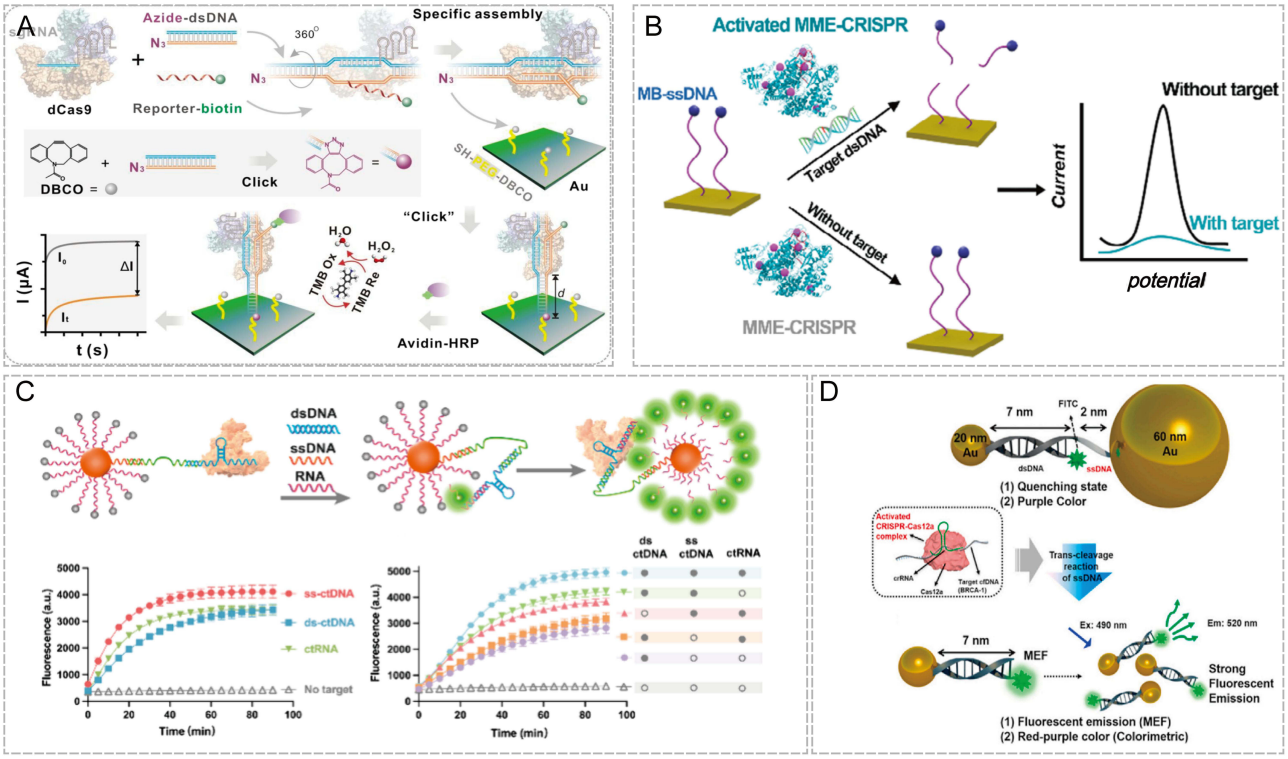

Figure 6 Integration of CRISPR/Cas systems with nanotechnology for ultrasensitive ctDNA detection. (**A**) dCas9 complexed with azide-modified dsDNA and biotin-reporter undergoes copper-free click chemistry with DBCO-modified gold electrode, achieving oriented immobilization. Target recognition induces 360° rotation, enabling specific assembly. Avidin-HRP conjugation catalyzes H_2O_2/hydroquinone redox cycling, generating amplified electrochemical current for mutant ctDNA quantification. This oriented covalent attachment via azide-DBCO click chemistry ensures optimal sgRNA accessibility and eliminates nonspecific adsorption, enabling single-nucleotide discrimination in plasma samples with femtomolar sensitivity. Reproduced with permission from reference.[50] Copyright 2023 American Chemical Society. (**B**) Metal-organic frameworks stimulate Cas12a trans-cleavage activity through surface-confined molecular crowding and metal-ion coordination effects. Upon target recognition, activated Cas12a cleaves methylene blue-labeled ssDNA probes on the electrode, releasing MB tags and generating measurable current reduction. Without target, probes remain intact with baseline signal. Differential pulse voltammetry shows distinct current responses. This MOF-CRISPR synergy amplifies electrochemical signal by ~100-fold compared to conventional Cas12a systems, achieving attomolar detection limits for low-abundance ctDNA without pre-amplification. Reproduced with permission from reference.[52] Copyright 2025 Wiley-VCH GmbH. (**C**) AuNP core decorated with radially oriented dsDNA, ssDNA, and crRNA strands forms Corona structure. Middle: Sequential target binding activates Cas12a trans-cleavage of fluorophore-quencher labeled reporters. Green fluorescent cluster formation indicates positive detection. Time-resolved fluorescence kinetics demonstrate dose-dependent responses for single-stranded ctDNA, double-stranded ctDNA, and circulating tumor RNA versus no-target control. The spherical AuNP architecture enables simultaneous presentation of multiple nucleic acid targets, achieving real-time multi-analyte detection within 100 minutes with picomolar sensitivity for comprehensive liquid biopsy profiling. Reproduced with permission from reference.[56] Copyright 2024 American Chemical Society. (**D**) 20 nm AuNP functionalized with 7 nm dsDNA spacer and 2 nm FITC-labeled ssDNA reporter (60 nm final diameter). Activated CRISPR/Cas12a complex binds target dsDNA, triggering trans-cleavage of ssDNA reporters. Cleavage releases FITC fluorophores from AuNP surface, eliminating quenching and enabling dual readout: molecular extinction fluorescence (MEF) at Ex. 480 nm/Em. 520 nm producing strong green emission, and colorimetric shift from red-purple to blue due to AuNP aggregation state changes. This plasmonic-fluorescent dual-signal architecture provides built-in cross-validation, achieving zeptomolar detection limits (10^{-21} (M) for cell-free tumor DNA with single-copy sensitivity, while the colorimetric component enables equipment-free visual screening. Reproduced with permission from reference.[58] Copyright 2021 Elsevier B.V.

Point-of-Care Testing for ctDNA: A New Frontier

Point-of-care platforms represent an emerging frontier in ctDNA detection. By integrating nanomaterial-based sensors with portable devices such as smartphone readouts, microfluidic chips, or paper-based assays,[59] POCT systems enable real-time, decentralized testing outside specialized laboratories. Recent advances in nanozyme-assisted colorimetric sensors and nanopore-based portable readers have demonstrated the potential to achieve rapid (<30 min), low-cost, and highly sensitive ctDNA detection at the bedside or in resource-limited settings. Such POCT innovations not only bridge the gap between laboratory research and clinical translation but also hold promise for early cancer screening, therapy monitoring, and personalized disease management in diverse healthcare environments.[60]

Downstream Workflow: Nanotechnology for Multidimensional Profiling

The detection of standard genetic alterations such as SNVs, indels, and gene fusions represents a traditional yet vital domain where nanotechnology-based platforms have made a profound impact. In recent years, fragmentomics and epigenomics have emerged as promising areas of research, offering new approaches for cancer detection. The application of nanotechnology in these two fields has opened a new era in non-invasive tumor diagnostics. Nanomaterials play a key role in enhancing ctDNA analysis, improving the enrichment of ctDNA fragments and the recognition of epigenetic markers such as DNA methylation. These advancements are transforming the landscape of ctDNA detection, providing more comprehensive and accurate tools for cancer screening. Table 2 compiles representative ctDNA detection applications across major cancer types and their clinical advantages.

Table 2 Nanotechnology Applications for ctDNA Detection Across Different Cancer Types

Cancer Type	Nanotechnology Application	Clinical Example/Advantage	Technology Enhancements	References
Breast Cancer	Fe_3O_4-Au core-shell nanoparticles, magnetic nanoelectrode–based electrochemical biosensor	A detection limit as low as 2.79 aM (~2 copies μL^{-1}) was achieved for ESR1 mutation analysis, enabled by a 20-fold electrochemical signal amplification through repeated measurement cycles.	Surface-confined amplification using dispersible magnetic nanoparticles significantly enhanced electrochemical signal intensity and reduced background noise.	[6,61]
Lung Cancer	Quantum superstructures for trace ctDNA detection and rapid diagnosis	Achieve 10^{-15} M detection limit with high reproducibility (RSD = 3–12%) and ≈95% diagnostic sensitivity in identification of ALK, BRAF, EGFR, KRAS, MET, NETRK1, enabling tissue-of-origin identification with >90% specificity	Nanomaterial-enhanced quantum superstructures offer extremely high detection sensitivity, even for early-stage cancer.	[5,40,62]
Hepatocellular Carcinoma	Au@Pt@HP1–HP2@Fe_3O_4 nano enzymatic complexes combined with catalytic hairpin assembly (CHA) and SERS technology	Achieve highly sensitive identification of PIK3CA E542K mutant ctDNA in serum, reaching a detection limit of 4.12 aM	The hybrid structure combines Au@Pt nanostructures for SERS enhancement and Fe_3O_4 nanobeads for magnetic enrichment	[63,64]
Colorectal Cancer	Gold nanoparticle–based multiplex lateral flow strip for ctDNA KRAS mutation analysis	Enable simultaneous analysis of four KRAS alleles on a single strip with 0.1% mutant allele frequency sensitivity, comparable to ddPCR, providing results within 15 min at significantly lower cost than NGS or ddPCR, offering a rapid and affordable solution for liquid biopsy–based CRC genotyping.	Integration of AuNP-labeled probes with multiplex primer extension and lateral flow readout enhanced visual signal clarity and detection throughput	[65]

(*Continued*)

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Table 2 (Continued).

Cancer Type	Nanotechnology Application	Clinical Example/Advantage	Technology Enhancements	References
Pancreatic Cancer	Metal–graphene hybrid terahertz (THz) meta surface sensor integrated with AuNP-assisted Hybridization Chain Reaction (HCR)	Enable enzyme-free and ultrasensitive detection of KRAS G12D ctDNA in pancreatic cancer plasma, achieving a detection limit of 0.22 fM and single-base mismatch discrimination.	The graphene–AuNP hybrid meta surface enhance THz charge transfer and optical resonance response, while magnetic bead–initiated HCR provides additional chemical amplification, resulting in dual signal enhancement and subfemtomolar-level detection precision.	[5,66]
Ovarian Cancer	DNA nanoswitch–based multi-channel biosensor integrating CHA and toehold-mediated strand displacement (TDSD)	Enable simultaneous detection of up to 10 ctDNA biomarkers relevant to early-stage ovarian cancer, specifically targeting high-frequency mutations in genes such as TP53, BRCA1and BRCA2. Incorporation of CHA reduced the limit of detection to 1.13–1.45 nM, improving sensitivity by 26–31 fold, and providing a scalable strategy for multiplex and high-throughput screening.	The modular DNA-nanoswitch architecture combines CHA and TDSD reactions to amplify fluorescence outputs with dual-signal readouts, achieving efficient, parallel analysis of multiple targets.	[5,67]
Prostate Cancer	Local electric potential–driven nanofluidic biosensor for ion transport–based ctDNA sensing	Enable highly specific detection of prostate cancer–related ctDNA in plasma such as ALK, BRAF, BRCA1 and BRCA2 mutation, achieving a 1 fM detection limit with a linear dynamic range of 10^{-15}–10^{-10} M. The approach demonstrated a >1000-fold sensitivity improvement over conventional resistance-based nanofluidic sensors, enabling clear differentiation between patients and healthy individuals.	The design exploits photoinduced carrier diffusion to generate localized membrane potential changes while maintaining constant system resistance, producing a gated ionic current modulation that enhanced both sensitivity and signal stability.	[6,68]
Multiple Cancers	Magnetic nanoparticle–based electrochemical biosensing platform for universal ctDNA detection	A versatile nanoplatform employing dispersible magnetic nanoparticles for ctDNA isolation and signal transduction, applicable to various malignancies such as gastrointestinal and breast cancers. The NanoRCS platform integrates nanopore technology with RCA-based signal amplification to enable multimodal cfDNA profiling, specifically targeting a broad panel of oncogenic drivers including TP53, KRAS, PIK3CA, BRAF, and ESR1, achieving reliable tumor-fraction detection down to 0.24%.	Integration of magnetic enrichment with electrochemical signal amplification allows noninvasive ctDNA analysis without requiring tumor tissue.	[6,69–73]

Epigenetic Profiling

Epigenomics studies how gene expression is regulated by reversible chemical modifications, such as DNA methylation and histone modifications, without changing the DNA sequence.[74] These regulatory changes are crucial in the development of diseases like cancer. By analyzing methylation markers in ctDNA, researchers can effectively detect early cancer and identify its subtypes.[75] Research demonstrates 26 obtained candidate ctDNA methylation biomarkers in breast cancer show 100% sensitivity and 75% specificity for early detection and treatment prediction.[76]

Application of Nanostructures and Nanosensors in ctDNA Epigenetic Detection

DNA nanostructures, such as DNA tetrahedra, G-quadruplexes, DNA hydrogels, and DNA origami, can enhance DNA methylation detection by integrating with isothermal amplification techniques like rolling circle amplification (RCA), catalytic hairpin assembly (CHA), and exponential isothermal amplified strand displacement reaction (EXPAR). This integration improves the efficiency of detecting methylation-related targets, such as DNA methyltransferase activity, and stabilizes amplification modules in methylation-focused assays.[21]

Nanomaterial-based sensors, particularly electrochemical and optical sensors, have shown great potential in the epigenetic detection of ctDNA. The unique physicochemical properties of nanomaterials enable customized detection techniques targeting the methylation patterns of ctDNA. Leveraging the specific affinity of gold nanoparticles for methylated ctDNA, a research team developed an electrochemical biosensor for the detection of early-cancer patients. This method employed square wave voltammetry (SWV) to distinguish between healthy and cancer samples based on cfDNA methylation differences, achieving a sensitivity of 0.89 and specificity of 0.73. The study revealed that ctDNA from cancer patients exhibited stronger absorption on gold surfaces compared to healthy controls, a difference closely related to the methylation status of the ctDNA, with nanomaterials' high surface area and selective binding properties providing key support.[77–79]

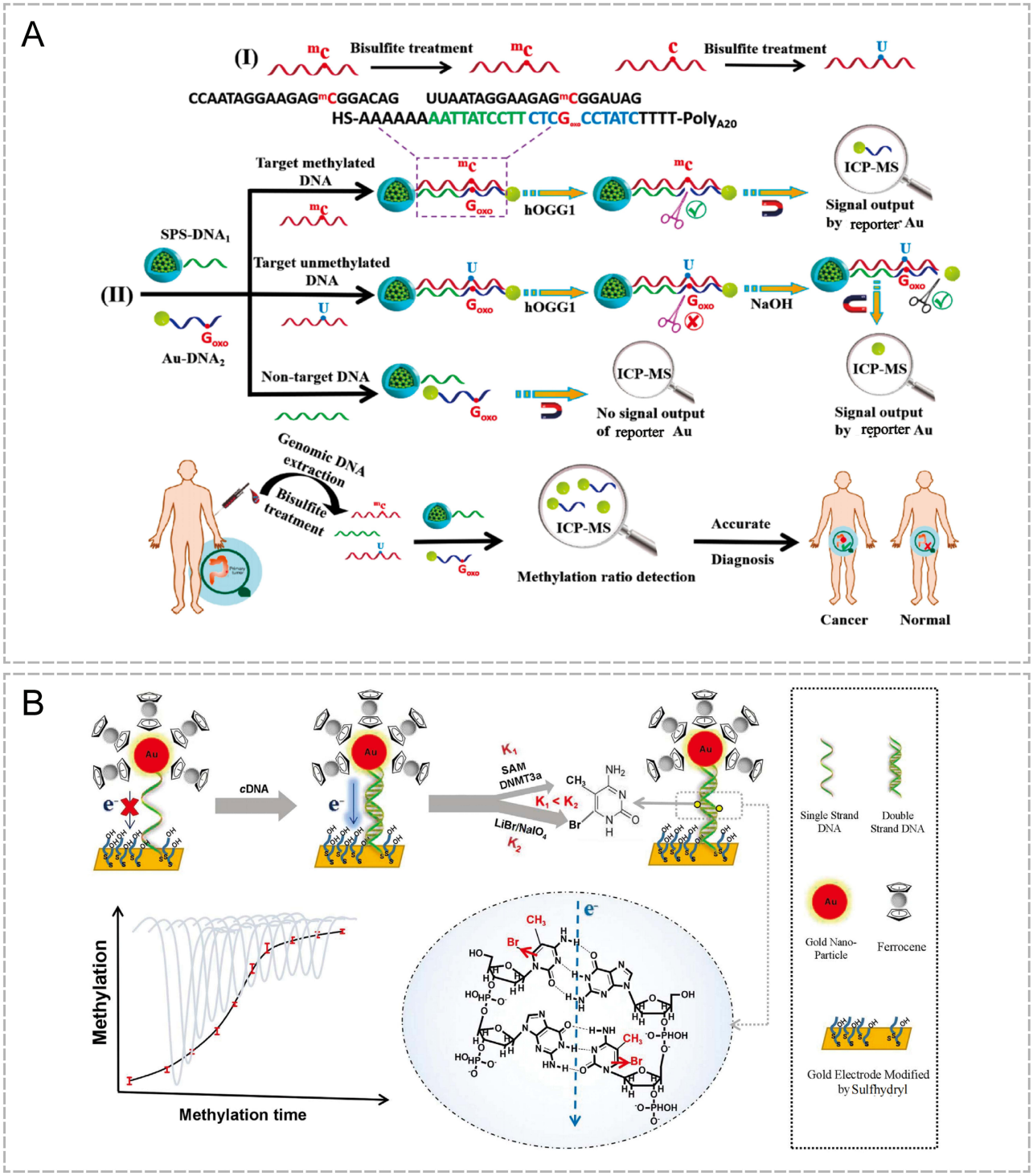
Additionally, nanoparticle-based sensing platforms, particularly dispersible magnetic nanoparticles, have emerged as powerful tools for the electrochemical detection of methylated ctDNA. By enabling in situ enrichment of target ctDNA on the Fe_3O_4-Au core-shell nanoparticle surface, these systems achieve ultrasensitive (≈3 aM) and rapid (≈7 min) detection of metastatic breast cancer–derived ctDNA.[6] Such platforms provide base-pair–level resolution, allowing precise discrimination of methylation states at specific CpG sites within the target sequences.[80] The remarkable analytical performance is largely attributed to the surface-enhanced properties of nanomaterials such as SPR and Raman scattering effects which amplify local electromagnetic fields and significantly boost the detection signal.[77] Integrating these nanostructures with defined panels of methylation biomarkers (such as a 15-marker signature yielding an AUC of 0.967 for early breast cancer diagnosis) has enabled high-accuracy cancer stratification.[75,76] These advances highlight the potential of nanoparticle-assisted electrochemical methylation assays as rapid, cost-effective, and minimally invasive tools for early cancer screening and monitoring of minimal residual disease (MRD), paving the way toward clinically applicable precision diagnostics in oncology.[81,82] Figure 7 summarizes nanotechnology-assisted strategies for ctDNA methylation detection.

Nanopore Applications in ctDNA Epigenetic Detection

Nanopore technology enables precise methylation detection by monitoring the current changes as DNA molecules pass through the nanopore. Single-molecule real-time sequencing eliminates the need for PCR amplification, thereby preserving native DNA features and allowing concurrent readout of genomic, epigenomic, and fragmentomic landscapes. It directly resolves cytosine modifications such as 5mC and 5hmC, while offering practical advantages including shorter turnaround time, lower analytical cost, and reduced DNA input load. This method offers noninvasive, highly sensitive and high-resolution detection, with significant applications in early cancer diagnosis and monitoring treatment responses.[85] By adjusting the pore size, MoS_2 monolayer nanopores can achieve label-free, single-nucleotide resolution methylation detection. Studies show MoS_2 nanopores can distinguish nucleotides differing by just one methyl group and directly identify multiple methylation site spaced as short as 70bp in double-stranded DNA.[86] This technology avoids the damaging modifications associated with traditional methylation detection methods, such as bisulfite treatment or antibody

enrichment, providing a more precise and efficient detection approach.[85] The overview of nanopore sequencing and conventional platforms for ctDNA methylation detection is illustrated in Table 3.

Beyond single-gene assays such as SEPT9, genome-wide methylation profiling using nanopore sequencing has shown strong diagnostic potential for breast cancer and other malignancies, enabling simultaneous detection of 5mC and 5hmC without bisulfite conversion. Concurrently, cfMeDIP-seq analyses have revealed that early methylome alterations during



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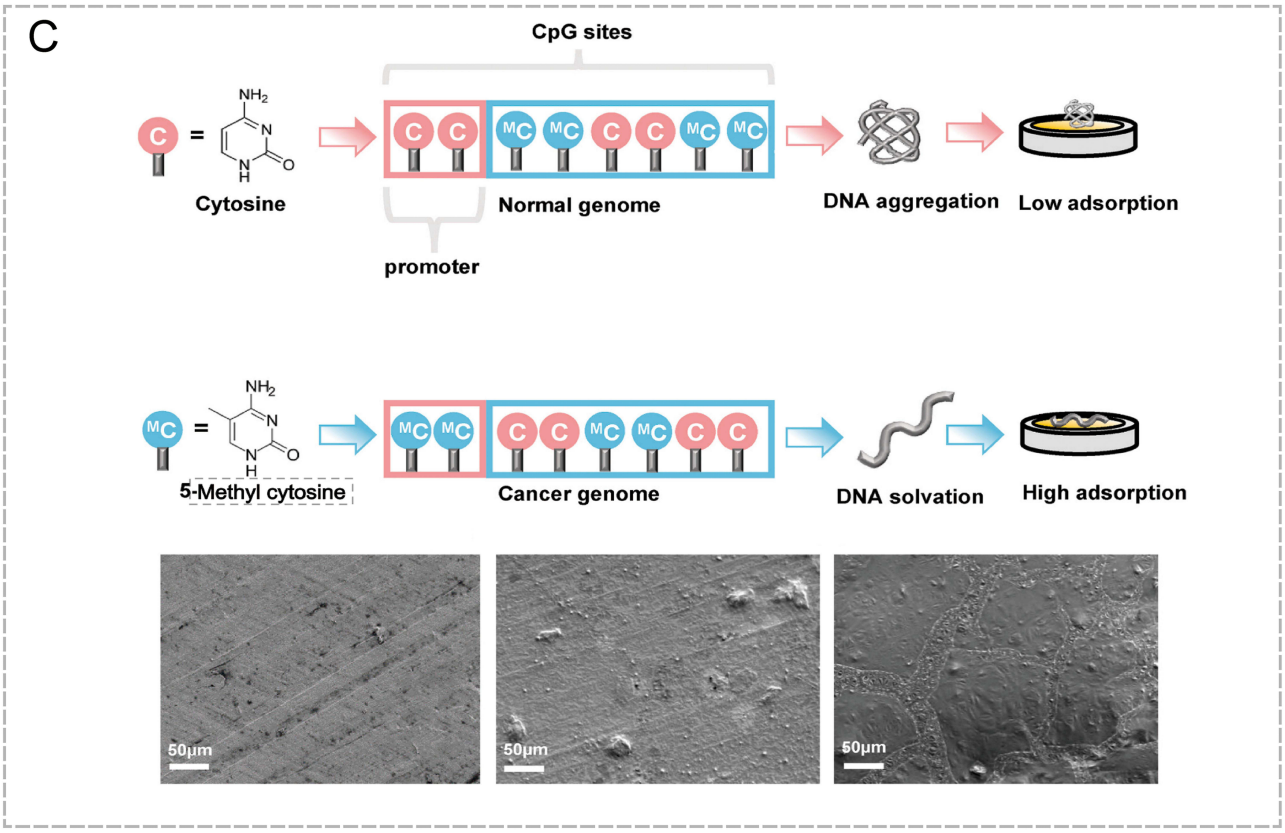

Figure 7 Nanotechnology-assisted strategies for ctDNA methylation detection. (**A**) Bisulfite treatment converts unmethylated cytosines to uracils while preserving 5-methylcytosines (5mC), generating distinct sequences for methylated versus unmethylated DNA. Target methylated DNA hybridizes with SPS-DNA_1 on gold nanoparticles, followed by 8-oxoGGGI-mediated oxidation at guanine sites and subsequent ICP-MS signal detection. Target unmethylated DNA undergoes similar capture but generates differential oxidation products via NaOH treatment, enabling methylation state discrimination. Non-target DNA produces no signal, while Aa-DNA_2 serves as internal control. Clinical workflow shows genomic DNA extraction from patient blood, bisulfite conversion, and ICP-MS-based methylation ratio quantification for accurate cancer diagnosis versus normal samples. This nanoparticle-triggered oxidation strategy coupled with ultrasensitive metal quantification achieves single-CpG resolution with attomolar detection limits, enabling precise methylation profiling without fluorescent labeling. Reproduced with permission from reference.[83] Copyright 2022 American Chemical Society. (**B**) Gold nanoparticle-modified electrode immobilizes ferrocene-labeled dsDNA probes via thiol-gold chemistry. Upon S-adenosylmethionine (SAM)-dependent DNMT3A catalysis, cytosine methylation alters DNA hybridization stability and electron transfer kinetics. Methylation progression curve demonstrates time-dependent signal evolution, with detailed mechanism shown in central circle: SAM donates methyl groups (CH_3) to cytosine, generating 5-methylcytosine and S-adenosylhomocysteine (SAH). Methylated dsDNA exhibits enhanced rigidity and reduced ferrocene-electrode distance ($K_1 < K_2$), facilitating electron transfer (e^-) and amplifying electrochemical current. Component diagram illustrates system elements: single-strand DNA probes, ferrocene redox mediators, AuNP-modified gold electrodes functionalized with sulfhydryl groups, and assembled dsDNA-ferrocene complexes. This label-free platform enables continuous, non-destructive monitoring of methyltransferase activity with sub-nanomolar sensitivity, providing mechanistic insights into epigenetic regulation and serving as a screening tool for DNMT inhibitors in cancer therapy. Reproduced with permission from reference.[84] Copyright 2017 Elsevier B.V. (**C**) Normal genome with unmethylated CpG sites in promoter regions exhibits weak affinity for gold surfaces, resulting in DNA aggregation in solution and low electrode adsorption. Cancer genome with hypermethylated CpG sites demonstrates enhanced hydrophobic interactions and stronger adsorption on gold surfaces, leading to DNA solvation and high electrode coverage. Scanning electron microscopy images confirm distinct surface morphologies: sparse DNA deposition from normal samples, moderate coverage from mixed samples, and dense DNA networks from cancer samples. This methylation-dependent adsorption behavior arises from altered electrostatic and hydrophobic properties of 5-methylcytosine versus cytosine, enabling direct discrimination without bisulfite conversion or chemical modification. The simple square wave voltammetry readout achieves 89% sensitivity and 73% specificity for early cancer detection, demonstrating clinical feasibility of rapid, low-cost methylation screening based on intrinsic nanoscale DNA-surface interactions. Reproduced with permission from reference.[79] Copyright 2025 Elsevier B.V.

therapy can predict clinical benefit and survival outcomes. These nanopore-based methylome readouts may be further integrated with tumor microenvironment–responsive nanodevices for upstream capture and downstream detection.[85]

Fragmentomics

Fragmentomics is a discipline that studies the fragment size, end motifs, end densities and nucleosome occupancy of cfDNA fragments across the genome, aiming to uncover their potential applications in early cancer detection.[97] Tumor-derived cfDNA typically exhibits distinct fragmentomic characteristics, including a shorter fragment length of approximately 143 base pairs, GC-rich terminal motifs such as GG or CC, and aberrant nucleosome protection patterns. These molecular features are closely associated with the chromatin organization, transcriptional activity, and cell death pathways of the originating tumor cells.[98]

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Table 3 A Comparative Overview of Nanopore Sequencing and Conventional Platforms for ctDNA Methylation Detection

	PCR-Based Methods	Traditional Bisulfite Sequencing	Methylation Array	Nanopore Sequencing	References
Sensitivity	High sensitivity for known loci, but limited coverage	Limited by long reaction times and low signal-to-noise ratio, low DNA input may lead to underestimation of methylation.	Moderate; detection threshold ≈ 1–5% methylation for most CpGs.	Extremely high; direct single-molecule methylation reading with sensitivity down to femtomolar levels using solid-state (SiN) or biological nanopores.	[87,88]
Specificity	High for targeted CpG sites; depends on primer design	Resolution based on CpG sites, may be affected by incomplete conversion and sequence bias.	High for predefined loci; platform-validated probe sequences minimize cross-reactivity.	Very high; capable of distinguishing 5-mC, 5-hmC and unmethylated C without chemical conversion, enhanced by ionic-current pattern recognition.	[89,90]
Ease of Operation	Relatively simple (qPCR, Methylation-Specific PCR), but requires optimization	Requires complex experimental processes including bisulfite conversion, PCR amplification, and sequencing.	Standardized workflow with commercial kits; minimal manual handling.	Moderate; requires nanopore setup and bioinformatic analysis but no bisulfite or amplification steps.	[91,92]
DNA Damage	Low, but amplification bias may occur	Bisulfite treatment often leads to DNA degradation	Moderate, bisulfite conversion induces some DNA degradation, but no mechanical fragmentation is required.	Minimal; sequencing native DNA preserves epigenetic integrity.	[91,93]
Detection Time	Hours (PCR cycles and analysis)	Typically requires several days, including DNA extraction, conversion, and sequencing	1–2 days; streamlined hybridization and imaging steps.	Rapid real-time signal acquisition, usually within hours.	[88,89]
Applicable Range	Suitable for targeted biomarker validation	Suitable for genomic research and methylation analysis, especially with higher DNA amounts.	Large-scale CpG profiling, disease-specific panels.	Genome-wide and single-molecule methylation profiling for low-input cfDNA and long-read haplotype analysis.	[76,94]
Technical Requirements	Standard PCR/ qPCR instruments	Highly dependent on complex equipment and strict environmental control during the process.	Microarray scanner and bioinformatics pipeline.	Can be integrated into portable devices, reducing the need for complex lab equipment, suitable for portable detection platforms.	[5,16]
Detection Accuracy	High for specific targets; lower for genome-wide	Accuracy decreases in low-abundance samples due to interference from background DNA	High reproducibility across samples; limited novel site discovery.	Single-molecule resolution, improved by machine-learning signal deconvolution.	[5,39]
Sample Volume Requirement	Moderate, depends on assay	Requires larger blood sample volumes for DNA extraction, suitable for high-abundance DNA samples.	Moderate; ~250–500 ng DNA input per array.	Requires minimal sample volume, particularly suitable for high-sensitivity detection of low-abundance ctDNA.	[5,95]

(*Continued*)

Table 3 (Continued).

	PCR-Based Methods	Traditional Bisulfite Sequencing	Methylation Array	Nanopore Sequencing	References
Cost-Effectiveness	Low cost per assay, scalable	High cost, involving expensive equipment and a complex laboratory workflow.	Moderate cost with large data output.	Moderate; low equipment cost and simplified workflow, but higher per-run consumable expenses.	[16,39]
Clinical Potential	High for clinical validation of known biomarkers	Widely used for cancer methylation analysis, but its application in ctDNA detection is limited due to standardization issues.	Extensively applied to population-level methylation epidemiology and cancer classification.	High potential for direct liquid biopsy methylation profiling and real-time diagnostics integration.	[5,96]

Nanopore sequencing has recently emerged at the intersection of nanotechnology and cfDNA fragmentomics. Fragmentomic profiling of plasma Epstein-Barr virus (EBV) DNA has demonstrated potential in cancer screening and risk prediction. In nasopharyngeal carcinoma (NPC) screening, this approach can predict future cancer risk (relative risk up to 87.1 times).[99] In the diagnosis of ovarian cancer (OC), fragmentomic features analyzed through shallow whole-genome sequencing have achieved high accuracy (AUC=0.97), surpassing traditional biomarkers such as CA125 and the ROMA index.[100]

Multi-Omics Integration

Nanotechnology has become a pivotal enabler of multi-omics and multi-parametric analysis in ctDNA research. By integrating diverse molecular signals such as genetic mutations, methylation states, and fragmentomic features onto unified nanoscale platforms, it enables more sensitive and comprehensive liquid biopsy. Multimodal nanotechnologies are being extended toward precise diagnosis and longitudinal disease monitoring.[14]

Key Enabling Roles of Nanotechnology in Multi-Omics Integration

Single-molecule multi-omics co-detection has been realized through nanopore devices. This configuration establishes a prototypical nano-driven multi-omics analytical workflow that is particularly suited for longitudinal monitoring. In shallow WGS modes, nanopore-based assays achieve concordance with Illumina WGS and WGBS while preserving complete fragmentomic resolution.[101,102]

Nanotechnology-assisted nucleic acid enrichment and signal transduction further enhance assay performance. Antibody or aptamer-functionalized magnetic and plasmonic nanoparticles markedly improve the signal-to-noise ratio (S/N) by selectively capturing rare variants and structural signals.[11,103] What's more, nanostructured systems, such as nanoelectrodes and DNA walker/origami-based circuits, translate subtle molecular events into amplified electrical or optical outputs, which can be deeply integrated with sequencing-derived data for multi-dimensional analysis.[68,104,105]

Multi-channel integrated sensing platforms represent another frontier of nano-enabled liquid biopsy. Dual-mode electrochemical–optical nanosensor arrays employ cross-validation mechanisms, such as fluorescence-based preliminary recognition followed by electrochemical quantification, to ensure analytical reliability. Such architectures are particularly advantageous for dynamic monitoring of therapeutic response, allowing precise capture of tumor burden fluctuations in real time.[68,105,106]

Clinical Applications of Nanotechnology and Multi-Omics Datasets Analysis

Single-omic assays such as those focusing solely on gene mutations or DNA methylation often show limited performance in low–tumor-fraction cfDNA backgrounds, particularly in MRD detection and early therapeutic response evaluation.[107,108] In contrast, multi-modal integration leverages orthogonal cfDNA dimensions, such as point mutations, CNVs, and fragmentation patterns, to provide complementary molecular information, thereby enhancing detection power

when signals are weak or stochastic. Ultra-low-pass whole-genome sequencing (ULP-WGS) combined with fragmentomic analysis can determine copy-number abnormalities and fragment patterns within less than 24 hours, enabling more accurate estimation of tumor fraction and distinction between disease states.[109,110] Specifically, in studies involving cancer patients, methods analyzing cfDNA fragment-end composition, size, and tumor fraction were able to detect 72% of cancer cases at 95% specificity.[110] Such integrative approaches can mirror the mutational and CNV landscape observed in tissue biopsies, improving the robustness of diagnostic assessment and longitudinal disease monitoring.[111]

With the advancement of nanotechnology, the enrichment efficiency of ctDNA has been substantially improved, enabling more reliable detection even at ultra-low concentrations. In parallel, the integration of multi-omics features has further enhanced the analytical robustness of MRD detection. Together, nanotechnology-driven ctDNA enrichment and multi-omics–based molecular profiling have significantly advanced MRD assays, reducing false-negative rates and improving sensitivity for early relapse monitoring in solid tumors.[14,81] A dispersible magnetic nano-electrode platform demonstrated the capability to identify ESR1 mutations in metastatic breast cancer patient plasma with an ultralow detection limit of ~3 aM, providing a rapid tool for therapeutic monitoring.[6] In pancreatic cancer, which is notoriously difficult to diagnose early, a graphene-AuNP hybrid metasurface sensor achieved enzyme-free detection of KRAS G12D mutations with a limit of detection of 0.22 fM, significantly enhancing diagnostic precision in clinical samples.[66] Furthermore, nanopore-based consensus sequencing has enabled reliable tumor fraction estimation down to 0.24% by integrating genomic and fragmentomic features, demonstrating broad utility for longitudinal monitoring across multiple cancer types.[69] In CRC patients, plasma ctDNA MRD assays applying AVENIO targeted sequencing to samples that jointly assess genomic and methylomic alterations achieved detection rates of 83% at baseline and 15% after surgery,[112] while MRD-positive individuals exhibited significantly shorter progression-free survival (6.4 vs 28.1 months).[112,113]

Nanotechnology-assisted multi-omics analysis enables real-time therapeutic evaluation via dynamic molecular changes. In targeted therapy for non–small cell lung cancer (NSCLC), dynamic monitoring of ctDNA allele frequency reveals that patients exhibiting rapid ctDNA clearance during early treatment achieve significantly prolonged progression-free survival (PFS; 8.3 vs 3.4 months; HR = 0.29, $p = 0.0007$), and this molecular response precedes radiographic assessment in predicting clinical benefit.[114,115] Dynamic alterations in ctDNA methylation and spatial proteomic signatures have further emerged as informative indicators of therapeutic response and residual disease biology. In a recent multi-omics investigation of metastatic ovarian cancer, combined surgical and blood-based MRD assessment revealed that patients with persistently positive ctDNA after first-line therapy exhibited markedly shorter overall survival (median OS, 32.4 months vs not reached; $p = 0.008$). In parallel, spatial proteomic profiling of MRD lesions identified the upregulation of hypoxia signaling and multiple actionable protein targets including CDK6, GLS, MSLN, and ERBB2, highlighting the potential of integrated ctDNA and proteomic analyses for improving prognostic precision and therapeutic stratification.[116] In NSCLC, postoperative ctDNA positivity has been associated with a significantly higher recurrence risk compared with ctDNA-negative cases (HR = 8.1; $p < 0.001$).[117] Regarding resistance surveillance, nanoscale ctDNA enrichment enables the detection of low-frequency resistance mutations, and longitudinal multi-omics profiling has identified accompanying PIK3CA mutations as key drivers of acquired resistance, with these patients exhibiting significantly shorter PFS.[118,119] Integrated nanotechnology strategies for multi-omics detection are illustrated in Figure 8.

Selection of Nanotechnology Based on the Detection Target

Selecting the optimal nanotechnology-assisted strategy requires aligning the detection platform with the specific clinical objective and molecular target characteristics. For the rapid, targeted identification of known somatic mutations such as EGFR or KRAS variants, signal-amplified biosensors utilizing CRISPR-Cas systems or electrochemical nanointerfaces are most effective due to their superior specificity and ability to achieve attomolar sensitivity. For epigenetic profiling where preserving DNA integrity is paramount, bisulfite-free approaches utilizing nanopore sequencing or nanomaterial-based affinity capture are preferable to avoid the degradation associated with traditional chemical conversion. When the diagnostic goal involves comprehensive fragmentomic analysis or tissue-of-origin localization, single-molecule nanopore platforms offer distinct advantages by simultaneously capturing native methylation marks and fragment length profiles without amplification bias. Ultimately, for challenging scenarios such as minimal residual disease monitoring where

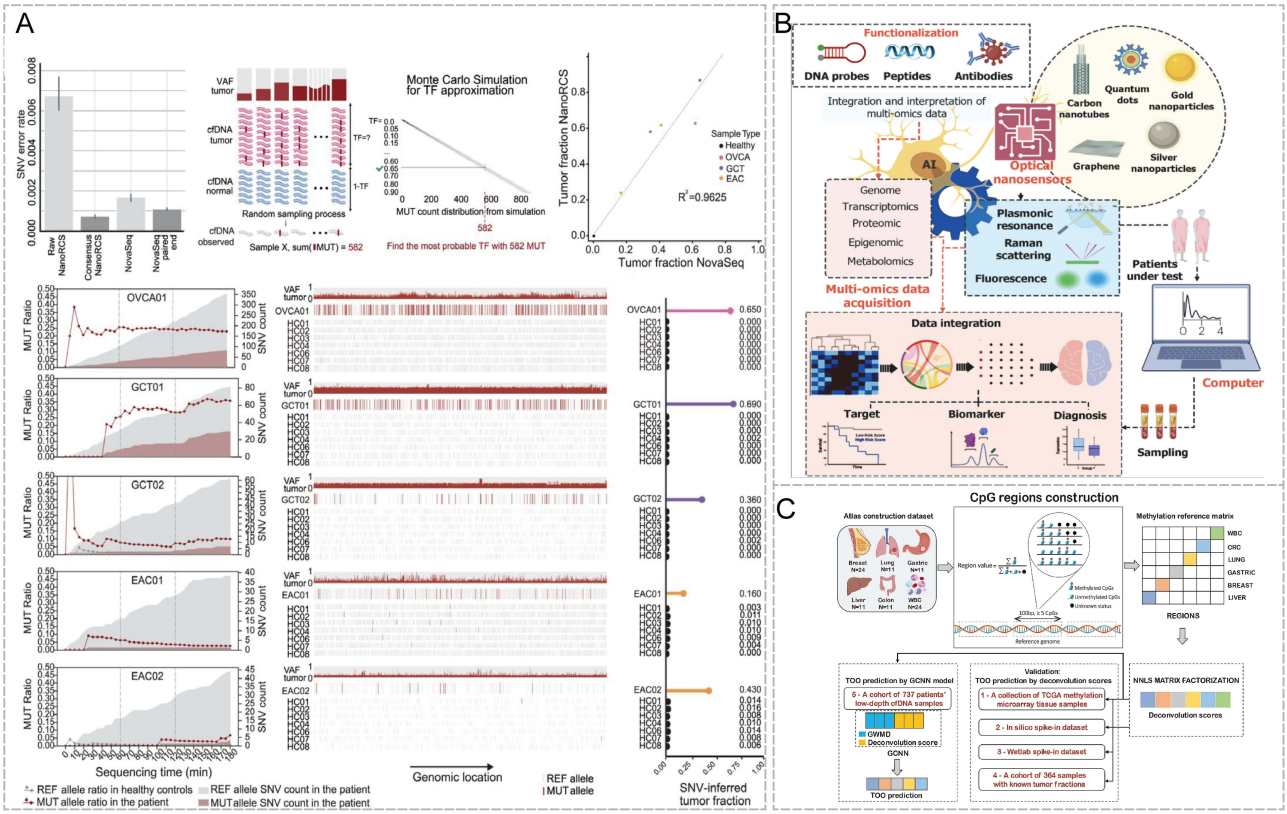

Figure 8 Integrated nanotechnology and AI-driven analytical strategies for multi-omics detection. (**A**) Nanoparticle-coupling and base-oxidation strategy. Bisulfite treatment preserves 5-methylcytosines while converting unmethylated cytosines to uracils. Methylated DNA (Route I) and unmethylated DNA (Route II) hybridize with SPS-DNA on gold nanoparticles, undergo differential oxidation (8-oxoGGGI or NaOH), and generate distinct ICP-MS signals. Clinical workflow shows DNA extraction, bisulfite conversion, and methylation ratio quantification for cancer diagnosis. This achieves single-CpG resolution with attomolar sensitivity. Reproduced with permission from reference.[69] Copyright 2025 Cold Spring Harbor Laboratory Press. (**B**) Ferrocene-labeled dsDNA on gold nanoparticle-modified electrodes undergoes SAM-dependent DNMT3A methylation. Methylation curve shows time-dependent signal evolution; central diagram illustrates CH_3 transfer from SAM to cytosine. Methylated DNA exhibits enhanced rigidity and improved electron transfer, amplifying electrochemical current. This label-free platform enables real-time methyltransferase monitoring with sub-nanomolar sensitivity. Reproduced with permission from reference.[120] Copyright 2025 Elsevier B.V. (**C**) Unmethylated CpG sites show weak gold affinity, causing DNA aggregation and low adsorption. Hypermethylated sites exhibit strong hydrophobic interactions and high electrode coverage. SEM images confirm sparse, moderate, and dense surface morphologies. This methylation-dependent adsorption enables direct discrimination without bisulfite conversion, achieving 89% sensitivity and 73% specificity for early detection. Reproduced with permission from reference.[121] Copyright The Author(s) 2024.

tumor fraction is ultra-low, a multi-modal integration strategy that synthesizes genomic variants with epigenomic and fragmentomic signatures is recommended to maximize signal-to-noise ratios and diagnostic reliability.

System Optimization: Integration with Artificial Intelligence

As nanoscale platforms continue to advance the sensitivity and dimensionality of ctDNA detection, the complexity and volume of molecular signals generated across these systems have increased correspondingly. Extracting biologically meaningful information from such heterogeneous datasets requires analytical frameworks capable of discerning subtle patterns, correcting for technical noise, and integrating multimodal features across genomic, epigenomic, and fragmentomic layers. Artificial intelligence provides a powerful computational counterpart to nanoengineered detection technologies, enabling the rational design of high-affinity molecular interfaces, the optimization of sensor architectures, and the interpretation of rich, high-dimensional outputs. Through deep-learning–based signal reconstruction and data-driven diagnostic modeling, AI offers new pathways to enhance system robustness, automate decision-making, and accelerate the translation of nano-enabled ctDNA assays into clinical practice.

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AI Optimized Nanoprobe Design

Artificial intelligence has fundamentally transformed the rational design and optimization of nanoprobes for ctDNA detection by enabling predictive modeling of molecular interactions and accelerating the discovery of high-performance recognition elements. Machine learning algorithms, particularly generative AI models and deep neural networks, can predict aptamer-target binding affinities, simulate conformational dynamics, and optimize sequence-structure-function relationships with unprecedented precision.[122,123] AI-driven aptamer design pipelines, integrating molecular docking simulations and structure-based modeling, successfully generate aptamer candidates with enhanced binding specificity while minimizing non-functional collapse on biosensor surfaces.[124] In nanomaterial synthesis, AI-powered robotic platforms employing Generative Pre-trained Transformer (GPT) models have achieved autonomous synthesis of diverse nanoparticles with controlled morphologies, demonstrating high reproducibility.[125] Furthermore, inverse design methodologies leveraging deep learning enable the optimization of plasmonic nanostructures for enhanced signal transduction, efficiently exploring vast design spaces that would be computationally prohibitive using traditional approaches.[126]

Deep Learning-Enhanced Multi-Omics Interpretation

A cascaded, layer-by-layer fusion strategy can be implemented based on nanopore sequencing technology (such as Oxford Nanopore technologies), whereby initial enrichment of variants and copy-number alterations enhances detection accuracy. Notably, CNV profiling can be achieved even at low sequencing depth, requiring as few as two million reads to identify cancer-associated genomic alterations.[127] For ambiguous or low–variant-allele-frequency (VAF) samples, additional layers of analysis can integrate fragmentomic features such as cfDNA fragment size distribution and end-motif patterns or methylation-derived indicators, including single-molecule methylome profiling, to stabilize variant calling and reduce interpretive uncertainty.[109] In the context of early treatment monitoring, the combined assessment of dynamic methylation changes and fragment-length shifts provides further clinical benefit; cfDNA methylome analysis has already been applied in longitudinal response evaluation of cancer patients, improving sensitivity for real-time disease monitoring.[128]

Building upon the multi-channel signals generated by nanopore sequencing platforms including genomic data such as VAF and CNV amplitude; fragmentomic features such as fragment-size spectra, end motifs, and nucleosome footprints; and methylation-based epigenetic information integrative modeling frameworks employ model-level multi-modal fusion strategies to converge these heterogeneous data streams into a unified classifier. Specifically, relation-aware multi-modal fusion modules can effectively capture intermediate-layer interactions between modalities, such as the interdependence between methylation signatures and fragmentation patterns, thereby maximizing the utilization of complementary information through joint feature-level and decision-level interactions.[129] In parallel, a coarse-to-fine dual-attention hierarchical architecture progressively aggregates cross-modal features, while a decoder-sharing mechanism enhances the sensitivity of the model to low-abundance ctDNA signals by reinforcing cross-domain consistency.[130] In multi-center validation datasets, deep-learning–based multi-modal fusion models, including graph convolutional networks (GCNs), have demonstrated significantly improved performance in tissue-of-origin deconvolution tasks, achieving 69% accuracy[121] and reducing misclassification rates to as low as two in 110 samples.[131] By jointly classifying single-molecule methylation states with fragmentomic features, these architectures enhance source attribution accuracy and clinical interpretability, thereby accelerating the translation of multi-modal ctDNA analysis into precision oncology.[128]

Conclusion and Future Perspectives

Conclusions

This review systematically summarizes and analyzes the complete workflow of ctDNA detection from the perspective of nanomaterials and nano–bio interfaces. It encompasses all critical stages, including nucleic acid capture and enrichment, signal amplification, single-molecule readout, multi-omics analysis, and the integration of artificial intelligence. Furthermore, the mechanistic roles of nanotechnology across each step of the workflow are elucidated, and a unified methodological framework is proposed. By establishing an integrated perspective spanning nanomaterials science, molecular diagnostics, machine learning and clinical applications, this review delineates recent technological advances

at the intersection of these disciplines. Through representative examples, we demonstrate how these nanotechnology-assisted strategies effectively lower the LOD and significantly improve the sensitivity and specificity of ctDNA assays.[50] Such advancements have paved the way for the clinical translation of liquid biopsy technologies, facilitating earlier cancer screening, real-time disease monitoring, and precision-guided therapy. Collectively, these developments highlight the promising yet evolving role of nanotechnology-driven multi-omics analysis in advancing personalized oncology, albeit with persistent challenges in clinical translation.[132]

While NGS and digital PCR continue to serve as the gold standards for discovering unknown variants due to their broad genomic coverage and quantitative precision, nanotechnology-based assays exhibit unique advantages for targeted mutation monitoring during therapy. These systems offer exceptional rapidity achieving analysis within minutes and ultra-high sensitivity at the attomolar to zeptomolar range, making them particularly suited for real-time, point-of-care applications such as tracking therapeutic resistance mutations.

Technical Direction

The foremost limitation lies in analytical sensitivity. ctDNA abundance in early-stage cancers is often below 0.01%, and although recent nano-enabled assays have achieved zeptomolar-level limits of detection as low as 10 zM, reported sensitivities vary widely across six orders of magnitude. Even with advanced signal amplification mechanisms, reliable identification of such rare variants still requires further optimization.[5] Moreover, matrix interference caused by non-specific adsorption of plasma proteins and urinary inhibitors remains a major source of analytical noise. Advanced antifouling surface modifications, such as polyethylene glycol (PEG) or zwitterionic coatings combined with refined sample pretreatment workflows are essential to ensure clinical-grade specificity.[133,134] In addition, multiplexing capability remains limited. In contrast to high-throughput NGS technologies capable of interrogating hundreds of genomic loci simultaneously, most nanobiosensing platforms can only target a few predefined mutations, such as EGFR or BRAF variants. The continued miniaturization and automation of nanosystems are therefore expected to enable true multi-analyte parallel detection optimized for specific molecular signatures.[135]

To overcome these limitations, the following avenues require concerted effort. Optical detection methods, particularly SERS, are also expanding beyond single mutation analysis toward comprehensive genomic and epigenetic profiling. Future large-scale studies are needed to establish robust correlations between SERS spectral fingerprints and specific genetic or epigenetic alterations. Enhancing the lattice architecture and surface chemistry of SERS substrates will further improve spectral resolution, allowing broader application across multiple cancer types, including those characterized by methylation-based biomarkers.

On the materials and engineering front, future development should prioritize novel nanomaterials with higher surface areas, tunable surface chemistries, and scalable, cost-effective synthesis routes to improve batch-to-batch reproducibility and reduce manufacturing costs. Concurrently, innovations in nanomaterial design such as bioinspired interfaces are expected to enhance ctDNA capture efficiency. Multifunctional nanoprobes capable of simultaneously detecting mutations, fragment length variations, and methylation states within a single assay will greatly increase molecular information integration. Emerging injectable or implantable nanosensors, such as DNA framework–based smart probes, represent another promising direction for real-time in vivo ctDNA monitoring and therapeutic response evaluation, addressing key limitations in sample stability and temporal resolution.[136]

In the field of electrochemical ctDNA biosensing, future efforts should emphasize precise control of the electrode–biomolecule–electrolyte interface to enhance reaction kinetics and reproducibility. Scalable fabrication of superparamagnetic nanomaterials will improve batch consistency. The integration of enzymatic catalysis and redox cycling may further enable cascade signal amplification for rapid and ultra-sensitive ctDNA quantification.[133]

AI Driven Optimization

Looking ahead, the convergence of nanotechnology with AI and multi-omics technologies is poised to fundamentally reshape the landscape of ctDNA detection. AI-driven analytical approaches markedly enhance diagnostic precision and enable dynamic tracking of molecular changes during therapy through optimized signal recognition and noise reduction. The integration of AI with molecular design tools is further driving the scalability of diagnostic systems. By applying

machine learning to optimize probe parameters and hybridization dynamics, it becomes possible to maintain ultra-high sensitivity while reducing assay cost, positioning nanochip-based systems as promising high-throughput diagnostic platforms. These advances leverage AI's pattern-recognition capabilities to refine multi-signal input and logic-output analyses, ultimately enhancing multiplexed molecular detection efficiency.[6]

Standardization and Regulation

Despite the transformative potential of nanotechnology in liquid biopsy, its translation from bench to bedside faces critical hurdles that cannot be overlooked. A primary concern is biosafety and biocompatibility; the high surface-to-volume ratio and chemical reactivity of nanomaterials, while advantageous for sensing, may induce unintended cytotoxicity, oxidative stress, or immune responses in biological systems, necessitating rigorous long-term nanotoxicology assessments.[137] Establishing international standardization frameworks for nanoplatform fabrication, characterization, and performance benchmarking will be essential to ensure safety, reliability, and clinical comparability.[138] Furthermore, manufacturing consistency remains a formidable obstacle to commercialization. Achieving batch-to-batch reproducibility in the synthesis of nanomaterials, specifically in controlling particle size distribution, surface charge, and ligand functionalization density, is essential for ensuring reliable clinical performance but is notoriously difficult to scale under Good Manufacturing Practice (GMP) standards.[139] These technical variability issues, coupled with a lack of harmonized regulatory frameworks for nano-bio interaction profiling, currently limit the widespread adoption of nano-enabled ctDNA assays in routine clinical practice.[137]

Another major bottleneck is the absence of standardized preanalytical and quality control frameworks. Harmonized protocols for specimen collection, storage, and DNA extraction, along with the establishment of key quality metrics such as ctDNA concentration thresholds and fragment integrity around 166 bp, are urgently required to ensure inter-laboratory comparability.[12,96] Furthermore, the downstream bioinformatic and interpretive frameworks demand rigorous standardization. From raw data preprocessing, quality filtering, and variant calling to clinical annotation and reporting, consistent pipelines are critical for achieving reproducible and interpretable results across laboratories. The establishment of evidence-based interpretation guidelines and signal-to-noise optimization strategies will be essential for the accurate identification of ultra-rare variants.

Clinical Translation

Although many nano-enabled ctDNA assays have shown strong analytical potential, their lack of clinical validation and regulatory standardization continue to hinder widespread adoption. Continued progress will require balanced optimization of performance metrics such as assay throughput, sequence coverage, and turnaround time through rigorous validation and harmonized guidelines.[96]

While nanotechnology-based enrichment strategies such as magnetic beads have been successfully integrated into regulatory-approved assays, the clinical translation of emerging sensing nanotechnologies of SERS, electrochemical, and CRISPR-nano platforms remains in its infancy. Unlike NGS-based panels including Guardant360 or FoundationOne which are supported by large-scale validation data from major clinical trials, most novel strategies are currently validated only in small-scale, single-center pilot cohorts, typically fewer than 100 participants. There is a notable absence of multi-center, prospective Phase III clinical trials evaluating the robustness of these nanodevices. Consequently, commercialized, regulatory-approved kits based purely on SERS or electrochemical principles are scarce. Future efforts should focus on moving from laboratory proof-of-concept to rigorous, large-scale clinical utility studies.

Overall, the pursuit of intelligent, scalable, and standardized nanotechnology holds the potential for robust ctDNA liquid biopsy, thereby strengthening its important role in early cancer detection, treatment monitoring, and precision-guided therapy in personalized oncology.

Data Sharing Statement

No primary research results, software or code have been included, and no new data were generated or analyzed as part of this review.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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