

Programmable DNA Aptamer-plasmonic Nanocomplexes for Biomedical SERS: Design Rules, Signal Transduction Architectures, and Translational Roadmap

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ABSTRACT: Surface-enhanced Raman spectroscopy (SERS) has emerged as a powerful platform for biomedical analysis, but its practical impact increasingly depends on how molecular recognition is translated into robust, programmable, and clinically compatible nanosystems. In this context, DNA aptamers act not only as biorecognition ligands, but also as programmable structural modules that define nanoparticle spacing, hotspot geometry, assembly dynamics, and signal-output logic. This review examines programmable DNA aptamer-plasmonic nanocomplexes as a design framework for biomedical SERS and organizes the field according to the architectural principles that govern analytical performance and translational potential. We classify current systems into linker-driven assemblies, conformational-switching platforms, catalytic and amplification-enabled networks, and higher-order addressable nanostructures, including DNA origami-based systems, and compare them across four design dimensions: sequence, topology, target-induced structural response, and signal-output logic. Unlike previous surveys organized primarily by target class or material, this design-centered framework enables architecture-performance trade-offs to be compared across systems beyond detection limits alone. We then highlight how these architectures shape sensing performance and biomedical applicability, while also examining the key barriers to clinical translation, including signal variability, limited standardization, aptamer instability, and manufacturing and regulatory constraints. Finally, we outline a translational roadmap for next-generation aptamer-SERS systems integrating reproducible plasmonic design, quantitative robustness, machine-learning-assisted analysis, and point-of-care compatibility. By connecting programmable architecture with signal transduction and translational readiness, this review provides a design-oriented perspective for the development of clinically relevant aptamer-SERS platforms.

1. INTRODUCTION

Surface-enhanced Raman spectroscopy (SERS) was first reported in the 1970s [1], and over the past decade it has matured into a mainstream analytical platform for biomedical research, built on the molecular fingerprint that Raman scattering provides [2–7]. The SERS effect arises when analyte molecules are positioned near nanostructured metallic surfaces, resulting in a dramatic amplification of their Raman signals. This enhancement makes it possible to detect characteristic vibrational modes, molecular “fingerprints”, even at ultra-low analyte concentrations. The signal amplification is attributed to two primary mechanisms: electromagnetic enhancement, driven by plasmon-induced intensification of the local electric field, and chemical enhancement, involving charge-transfer interactions between the molecule and the metal surface [8,9]. Electromagnetic amplification occurs when metal nanoparticles, typically smaller than the wavelength of incident light, interact with electromagnetic radiation, causing a collective oscillation of conduction electrons known as localized surface plasmon

resonance (LSPR) [10,11]. This plasmonic resonance amplifies the local electric field, which enhances the polarizability of nearby molecules and increases the probability of inelastic light-scattering events [12,13]. Particularly strong enhancement occurs within confined regions between nanoparticles or at sharp geometrical features, known as hotspots, where the electromagnetic field is highly concentrated [14–18]. The intensity and reproducibility of these hotspots depend on several critical parameters, including the size, shape, composition, and spatial distribution of the metallic nanostructures, which are discussed in detail in Section 2. Accordingly, the analytical performance of SERS systems is governed not only by the intrinsic optical properties of plasmonic materials, but also by the extent to which nanoscale architecture can be programmed to control hotspot formation and signal reproducibility. Two principal strategies are commonly employed to fabricate such nanostructures: top-down and bottom-up approaches. Top-down methods, like nanolithography, allow precise patterning but often lack scalability. In contrast, bottom-up approaches rely on the chemical synthesis and self-assembly of colloidal nanoparticles, offering greater scalability but often facing challenges in uniformity and reproducibility. Between

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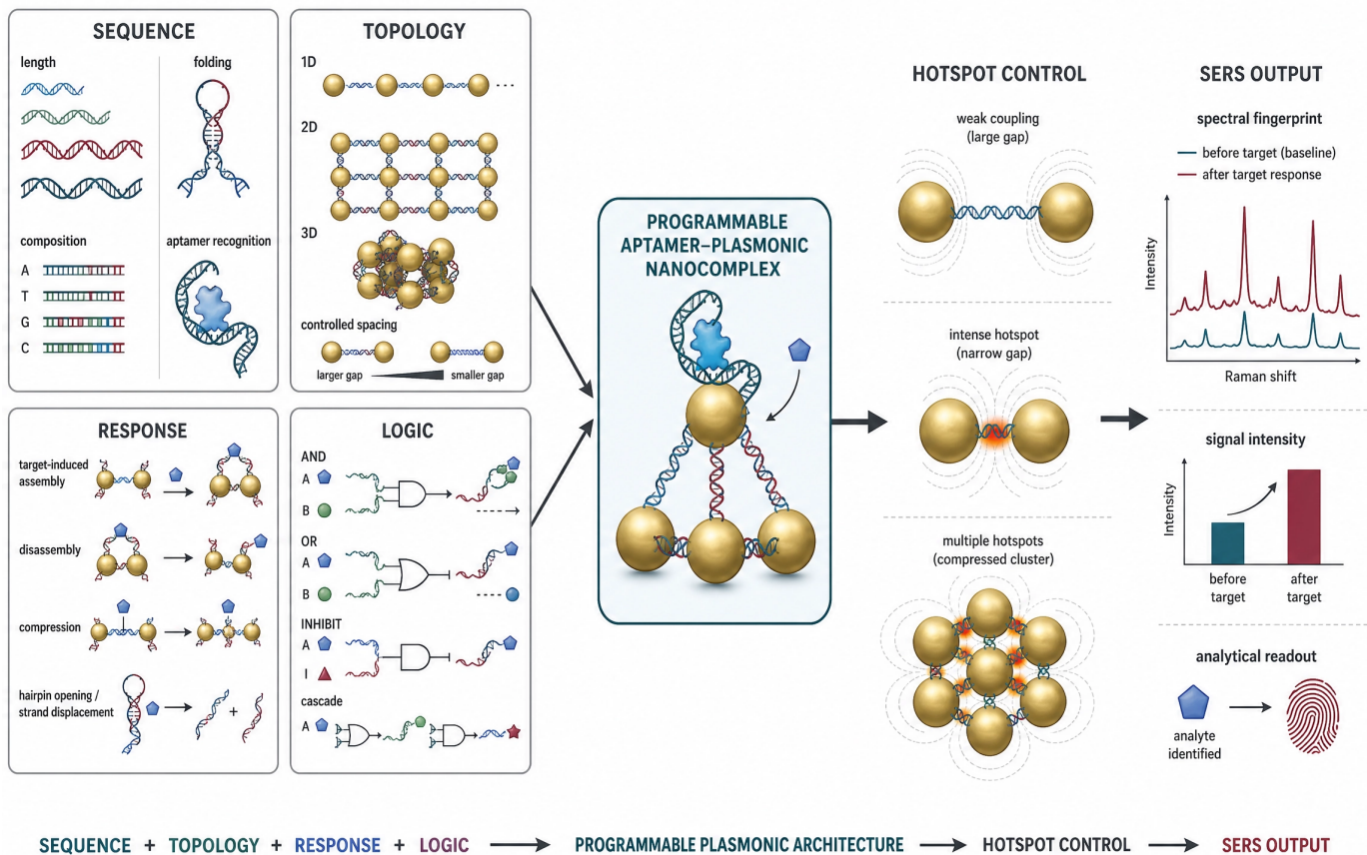


FIGURE 1. Programmable aptamer-plasmonic architectures integrate DNA sequence, topology, stimulus response, and molecular logic to regulate nanoparticle spacing, hotspot formation, and the resulting SERS output.

these approaches, emerging hybrid and template-assisted fabrication strategies, including template-assisted colloidal printing and guided nanoparticle assembly, provide a useful balance between structural uniformity, fabrication throughput, and cost. By combining predefined templates or patterned interfaces with solution-processable nanoparticles, these methods can reduce assembly variability while retaining compatibility with large-area and potentially flexible substrates. Such intermediate fabrication routes are particularly relevant for translational SERS, where reproducible performance must be achieved without relying exclusively on complex and expensive lithographic processes. By optimizing these fabrication techniques, it is possible to engineer nanostructures that generate intense local electromagnetic fields, thereby boosting Raman signals by factors as high as $\sim 10^{14}$ [19–22]. This extraordinary sensitivity enables SERS to detect a wide array of analytes, including environmental pollutants, pharmaceutical compounds, and biological markers, at concentrations down to the single molecule level [19, 23, 24].

Parallel to the advancements in plasmonic nanostructures, DNA aptamers have garnered increasing attention in biosensing applications [25, 26]. Aptamers are short, single-stranded oligonucleotides (ssDNA) selected through an *in vitro* evolutionary process known as Systematic Evolution of Ligands by

Exponential enrichment (SELEX) [27–29]. They enable high-affinity and high-specificity recognition of a wide variety of targets, including small molecules [30–34], peptides [35–40], proteins [26, 41–44], ions [45–53], and even whole cells [54, 55]. In contrast to traditional antibodies, aptamers exhibit superior chemical stability, lower immunogenicity, ease of chemical modification, and greater versatility in design [56, 57]. Aptamers can undergo structural changes upon target binding, such as hairpin opening, G-quadruplex formation, or duplex destabilization, making them particularly attractive not only as molecular recognition elements but also as programmable structural motifs for nanosystem engineering [58–61]. These complexes often act as scaffolds or linkers that control the spatial arrangement of SERS-active nanostructures, such as nanoparticle dimers, trimers, or aggregates, which directly influences the formation and location of SERS hotspots [62–64]. In this way, aptamers can be used to couple molecular recognition with nanoscale structural organization, enabling SERS platforms in which hotspot geometry, assembly dynamics, and signal output are all encoded at the sequence and architecture levels. By leveraging these properties, researchers can design modular and responsive SERS assays that produce strong signals in the presence of specific analytes. For instance, sandwich-type complexes can be formed between two nanopar-

ticles via aptamer–target–aptamer formation, enabling highly localized and more reproducible hotspots, with detection limits reaching the femtomolar or even single-molecule level [65–69]. Moreover, aptamer-mediated plasmonic nanocomplexes allow the detection of subtle molecular differences that may critically impact biological function, such as single-nucleotide mismatches, splice variants, or isoforms [70–73]. The high structural resolution and target selectivity of aptamers, when coupled with SERS, provide a unique approach to probe fine conformational changes and biochemical interactions in complex biological environments.

Despite these advances, the field is still too often framed primarily in terms of sensitivity enhancement, while the broader design rationale of aptamer-SERS systems remains underemphasized. The performance and translational potential of these platforms depend not only on target affinity or plasmonic amplification alone, but on how molecular recognition is integrated with programmable nanocomplex architecture, controllable signal transduction, and analytical robustness. This review therefore examines programmable DNA aptamer-plasmonic nanocomplexes as a design framework for biomedical SERS, rather than simply as combinations of recognition elements and signal enhancers. We organize the field according to the architectural and functional principles that govern hotspot formation, target-responsive reconfiguration, and sensing output. First, we examine how aptamers act as programmable structural modules for the assembly of 1D, 2D, and 3D plasmonic nanostructures with defined spatial organization. Second, we discuss the principal signal-transduction architectures enabled by aptamer binding, including conformational switching, target-induced assembly and disassembly, and amplification-coupled strategies. Third, we connect these design strategies to biomedical applications spanning nucleic acids, small molecules, proteins, extracellular vesicles, and live-cell systems. Finally, we assess the key barriers to translation, including signal reproducibility, aptamer stability, matrix effects, standardization, and regulatory constraints, and outline a translational roadmap for next-generation aptamer-SERS platforms integrated with quantitative design, machine-learning-assisted analysis, and point-of-care compatibility.

Notably, “programmable” is defined as the ability of DNA aptamers to encode, via their sequence and folded structure, the spatial organization of plasmonic nanostructures, the dynamic reconfiguration upon target binding, and the logic of signal transduction, allowing SERS output to be rationally designed rather than empirically optimized. To capture the design space of existing systems, we propose a programmable classification framework along four dimensions: (1) sequence (base composition, length, secondary structure), (2) topology (1D linker, 2D tile, 3D origami), (3) response (assembly, disassembly, compression, strand displacement), and (4) logic (Boolean AND/OR/INHIBIT, cascaded amplification). Each dimension can be assigned to a complexity level from L1 (single, static function) to L4 (multidimensional, conditional, and reconfigurable). Critically, “programmable” should be distinguished from related terms: “tunable” refers to continuous adjustment of a parameter (e.g., gap distance via DNA length) without

changing the system’s architecture or logic; “responsive” indicates a one-to-one structural change upon a single input (e.g., hairpin opening) but does not imply multi-state encoding or logic gating. Programmable systems therefore combine tunable parameters, responsive behaviors, and logic-coded reconfiguration into a unified design framework (Fig. 1).

2. PROGRAMMABLE DNA APTAMER-PLASMONIC NANOCOMPLEXES: DESIGN PRINCIPLES AND BIOMEDICAL FUNCTIONS

The integration of DNA aptamers with plasmonic nanostructures has enabled the emergence of programmable nanocomplexes in which molecular recognition, nanoscale organization, and signal generation are tightly coupled. In these systems, aptamers do not function solely as target-binding ligands; they also act as structural and dynamic elements that define interparticle spacing, guide nanoparticle assembly, regulate hotspot formation, and control signal-output pathways. As a result, the analytical performance of aptamer-SERS platforms depends not only on target affinity or plasmonic enhancement alone, but on how nanocomplex architecture is designed to translate molecular events into robust and tunable optical responses. In this section, we organize the field according to the core design principles that govern programmable aptamer-plasmonic systems, from structural assembly and target-responsive reconfiguration to signal transduction architectures and the biomedical functions they enable.

2.1. Aptamers as Programmable Structural Modules for Plasmonic Assembly

Aptamers have shown potential in fabricating nanosubstrates and advanced biosensing interfaces, particularly through the formation of target-driven biomolecular complexes [74, 75]. In aptamer-SERS platforms, however, their role extends beyond target recognition alone. Aptamers can function as programmable structural modules that direct the assembly, spacing, and dynamic organization of plasmonic nanoparticles, thereby governing hotspot formation and signal reproducibility. Typically, aptamers contribute to SERS biosensing in two principal ways: (1) as programmable linkers that mediate the assembly of nanoparticles into architectures capable of producing strong and localized electromagnetic (EM) fields, and (2) as molecular recognition elements that bind specific targets and induce structural organization [76, 77]. This dual role makes aptamers particularly valuable for engineering nanocomplexes in which molecular recognition and nanoscale optical function are intrinsically coupled.

Throughout this review we distinguish two roles that DNA plays in these systems. Structural DNA, meaning linkers, junctions, tiles and origami scaffolds, sets topology and interparticle geometry; an aptamer is a SELEX-derived sequence whose folded tertiary structure performs molecular recognition. Several of the architectures discussed below are more accurately described as DNA-programmed plasmonic systems containing an aptamer than as aptamer assemblies, and we use that wording where the DNA is structural.

1D Linker-based Assemblies. DNA-aptamer structures exhibit flexible nanomechanical behavior akin to that of soft 1D polymeric linkers. When two complementary DNA strands hybridize in an antiparallel orientation, they form a more rigid hybrid helix with a well-defined structural conformation. This strategy was pioneered by Faulds's group (Fig. 2(a)) [78] and was further developed by Nie's group, which showed that tuning the 1D sequence length, base composition, and orientation of DNA linkers on nanoparticle surfaces allows controlled regulation of cluster size, morphology, and interparticle spacing. Given that the distance between base pairs in double-stranded DNA (dsDNA) is approximately 0.34 nm, linker strands of 20–30 bases can be designed to give interparticle gaps of roughly 7–10 nm [79], although the spacing realized in practice also depends on grafting density and ionic strength, a regime favorable for strong plasmonic coupling and hotspot formation. This is especially advantageous for detecting short nucleic acid targets such as microRNAs or gene fragments [80, 81].

Duplex length is only one of three variables that set the realized gap. Grafting density determines how many strands compete for the same curved surface, and the maximum loading achievable depends on salt concentration, spacer composition and particle size rather than on sequence alone [82, 83]. Ionic strength then acts twice over: it screens the polyanionic backbone, setting both colloidal stability and the extent to which the DNA shell is compressed, and the effect is stronger for divalent than for monovalent counterions, with trivalent species collapsing the shell entirely [84, 85]. Linker flexibility completes the picture. Longer duplex interconnects give proportionally larger spacings but progressively poorer lattice order, which is attributed to the greater conformational flexibility of longer interconnects [86]. A programmable gap is therefore a designed gap only when grafting density and buffer conditions are specified alongside the sequence, which is one reason that reported interparticle distances are often less reproducible between laboratories than the underlying geometry would suggest.

Despite these advantages, these simple 1D structures inherently suffer from limited structural rigidity and environmental stability. Variations in temperature, pH, or ionic strength can destabilize the double helix, leading to dissociation of the aptamer linker and loss of nanoparticle aggregation, consequently reducing signal stability and analytical reproducibility. To overcome these limitations and extend the length of DNA linkers, rolling circle amplification (RCA) has been employed to synthesize long DNA strands containing repeated sequence motifs complementary to the nanoparticle-bound oligonucleotides [87, 88]. RCA-produced nanoarray provides a dense array of binding sites for plasmonic nanoparticles, increasing both the density and uniformity of nanoparticle organization. This method enables the construction of nanoarrays with controlled spatial distribution of plasmonic features, offering better sensitivity against low-abundance biomolecules. More broadly, such 1D systems illustrate a key design principle in programmable aptamer-SERS nanocomplexes: sequence-encoded control over interparticle distance can be used to tune optical coupling and sensing output.

2D Addressable Assemblies. Beyond linear linker systems, the modularity and tunable hybridization behavior of aptamer-plasmonic assemblies provide a conceptual and structural foundation for the construction of more robust 2D nanocomplexes [89]. The first breakthrough in the early 2000s was the creation of branched DNA motifs. In 2003, Dan's group introduced a method to synthesize Y-shaped DNA junctions and higher-order dendrimer-like structures with excellent modularity and extensibility (Fig. 2(b)) [90]. These motifs represent some of the smallest and most adaptable DNA-based frameworks capable of supporting multivalent functionalization. By functionalizing Y-shaped DNA with AuNPs, researchers have constructed well-defined nanoparticle trimers, where the DNA junction acts as both a connector and a geometrical template, facilitating the formation of ordered 2D assemblies. Expanding on this concept, dendritic assemblies derived from Y-shaped DNA have been used to orchestrate larger AuNP clusters with increasing complexity and interparticle connectivity. These assemblies provide a versatile platform to guide nanoparticle placement in quasi-planar orientations, enhancing both structural integrity and material tunability. Further evolution of these 2D systems led to the design of core-satellite nanostructures. For example, Xu's group demonstrated a two-layer hierarchical core-satellite SERS configuration driven by DNA hybridization through Y-shaped constructs formed from three complementary oligonucleotides. This method enabled reproducible spatial arrangement of nanoparticles around a central core, providing a more controlled route toward hierarchical hotspot engineering in two-dimensional architectures [91]. The development of DNA origami in 2006 further revolutionized 2D nanoscale design. Unlike traditional DNA motifs assembled via laborious, stoichiometrically sensitive methods, DNA origami uses a long scaffold strand typically derived from viral DNA that is folded into precise 2D shapes using hundreds of short "staple" strands in a single-pot reaction [92, 93]. These origami platforms offer nano-level addressability and excellent structural fidelity. By attaching aptamer-functionalized nanoparticles to specific binding sites on the origami via complementary sequences, researchers can precisely dictate nanoparticle positioning across 2D planes [92]. Ren's group used ribbon-shaped DNA nanostructures, assembled from repeating five-strand DNA tiles, as programmable substrates for the formation of plasmonic 2D metamaterial [94]. By customizing capture strands on triangular nanostructures, Chao's group guided the assembly of gold nanocubes (AuNCs) into face-to-face dimers with defined nanogap spacing, enabling configuration-specific SERS behavior [95]. These studies highlight how addressable DNA architectures shift aptamer-SERS design from stochastic nanoparticle aggregation toward predictable plasmonic organization.

3D Programmable Nanocomplexes. Recently, higher-order programmable architectures have extended this concept from planar assemblies to three-dimensional nanocomplexes with greater spatial precision and functional complexity. For example, Bald's group introduced a DNA origami 3D nanofork antenna, where a G-quadruplex aptamer was used to position single hemin molecules at a plasmonic hotspot in a non-

contact mode, allowing single-molecule SERS detection and electronic state characterization [96]. Extending into biomedical imaging, Barman's group has recently developed SPEC-TRA, an aptamer-integrated DNA origami platform carrying vibrational tags in the Raman-silent window, for targeted cancer cell imaging, demonstrating selective binding and signal readout via SERS in live-cell environments [97]. These examples illustrate that programmable aptamer architectures can support not only ultrasensitive detection, but also spatially resolved sensing and imaging in biologically relevant settings. For example, the assembly of Au nanopyramids from four DNA strands functionalized with AuNPs illustrates how aptamer-embedded systems can be engineered to respond dynamically to environmental cues, such as the presence of proteins or small molecules (Fig. 2(c)), triggering either the disassembly or conformational rearrangement of the 3D-nanostructure [98–100]. In this regard, a critical breakthrough in DNA nanotechnology occurred in 2009, when DNA origami advanced from planar 2D designs to 3D frameworks [101]. The enhanced structural complexity of 3D DNA origami has enabled the fabrication of sophisticated plasmonic assemblies with precise nanoparticle placement and controllable interparticle distances. Liedl's group demonstrated the utility of these 3D DNA frames with SERS by inducing optothermal shrinking of frame-like DNA origami scaffolds between AuNP dimers, achieving nanoscale gap sizes below 2 nm [102]. Such precise control over nanoparticle spacing was facilitated by the ability of DNA origami to act as a rigid scaffold that spatially constrains attached aptamer modules and plasmonic nanoparticles. This level of control is crucial for designing aptamer-functionalized 3D complexes, where the spatial organization of recognition aptamers and plasmonic components can be tailored to optimize binding efficiency and signal transduction [103, 104]. Expanding the complexity of aptamer-guided 3D nanoassemblies, Wang's group utilized DNA origami hexagon tiles as platforms to anchor six 10 nm AuNPs on their exterior surfaces, followed by in situ silver shell growth to produce Ag@Au hexagonal metamolecules [105]. The DNA-guided assembly enabled precise nanoparticle positioning and size control, while the incorporation of aptamers on the nanostructure surfaces provides target-recognition capability and transforms these metamolecules into multifunctional sensing units. Furthermore, through hierarchical assembly strategies, such as linking individual DNA origami tiles into dimers, trimers, or higher-order clusters, programmable and responsive 3D aptamer-plasmonic complexes can be fabricated, allowing the fine-tuning of nanoscale architecture and molecular recognition sites to suit diverse biosensing applications.

Taken together, these examples show that aptamers are not merely surface ligands appended to plasmonic materials, but sequence-programmable building blocks that define the spatial logic of nanoparticle assembly across one-, two-, and three-dimensional architectures. This structural programmability is central to controlling hotspot geometry, minimizing stochastic variability, and creating nanocomplexes whose sensing behavior can be rationally designed rather than empirically optimized. The SERS EF depends on a small set of structural parameters that DNA can encode: interparticle gap (G), particle size (D)

and arrangement symmetry (S). The gap dominates. Correlated structural-optical measurements on individual dimers and small clusters show that, for hotspot-containing particles, EF tracks gap size while remaining uncorrelated with aggregation state or plasmon resonance wavelength, with an EF spread of less than two orders of magnitude across a structurally diverse set of nanoantennas [106]. The scaling is steep but bounded: measured dependences range from inverse-exponential to approximately $G^{-2.4}$, and the experimentally determined dimer-to-monomer EF ratio on a solid substrate reaches only about 4.5 at a gap near 1 nm, even where the corresponding Raman intensity ratio reaches about 9 [107, 108]. Enhancements of 10^{10} and above are obtained for gaps below 0.5 nm and for slightly fused particles, and come from electromagnetic simulation rather than measurement [109]. Ordered architectures matter less for peak enhancement than for the width of the EF distribution. DNA-programmed nanogap particles place more than 90% of individual particles above 1×10^8 , narrowly distributed between 1×10^8 and 5×10^9 , a spread of about a factor of fifty, and with a linear concentration response; an ordered nanowire array gives a relative standard deviation of 14% for double-stranded DNA across more than four thousand measurement points, and 7–16% in a separate multiplexed measurement [110]. The design conclusion is therefore one of reproducibility rather than magnitude: sequence-encoded linker length sets G (approximately 0.34 nm per base pair plus spacer) [93] and topology sets symmetry, sequence also sets D, since DNA-directed overgrowth of gold nanorod seeds tunes particle geometry and shifts the plasmon resonance from the visible into the NIR-II region [111]. DNA-programmed assemblies therefore narrow the spread of optical output rather than simply raising its mean.

2.2. Molecular Recognition and Target-Responsive Reconfiguration

Assembly- and Proximity-driven Reconfiguration. Aptamer-target binding is not a static event but is often accompanied by conformational transitions that modulate the sensing assembly. In programmable aptamer-plasmonic nanocomplexes, molecular recognition is, therefore, inseparable from structural reconfiguration: target binding can reshape the nanosystem, alter interparticle spacing, redistribute reporters, and ultimately redefine the optical response. A typical example is a sandwich-type assay in which the binding of a target induces aptamer folding that brings reporter-labeled nanoparticles into close proximity with the substrate-bound particles, forming nanogaps of < 10 nm, where SERS enhancement is maximized (Fig. 3(a)) [112–114]. In such systems, the target acts not only as an analyte but also as a structural trigger that converts recognition into hotspot-generating assembly. As recently reported by Qu's group in a dual-mode PEC-SERS biosensing platform for the IBD-associated biomarker MMP-9, aptamer binding served as the initiating event for a target-responsive complex that enabled downstream detection of a second biomarker, IAP, through CRISPR-Cas12a-mediated cleavage. Upon target recognition, the system triggered the release of methylene blue as a reporter, allowing

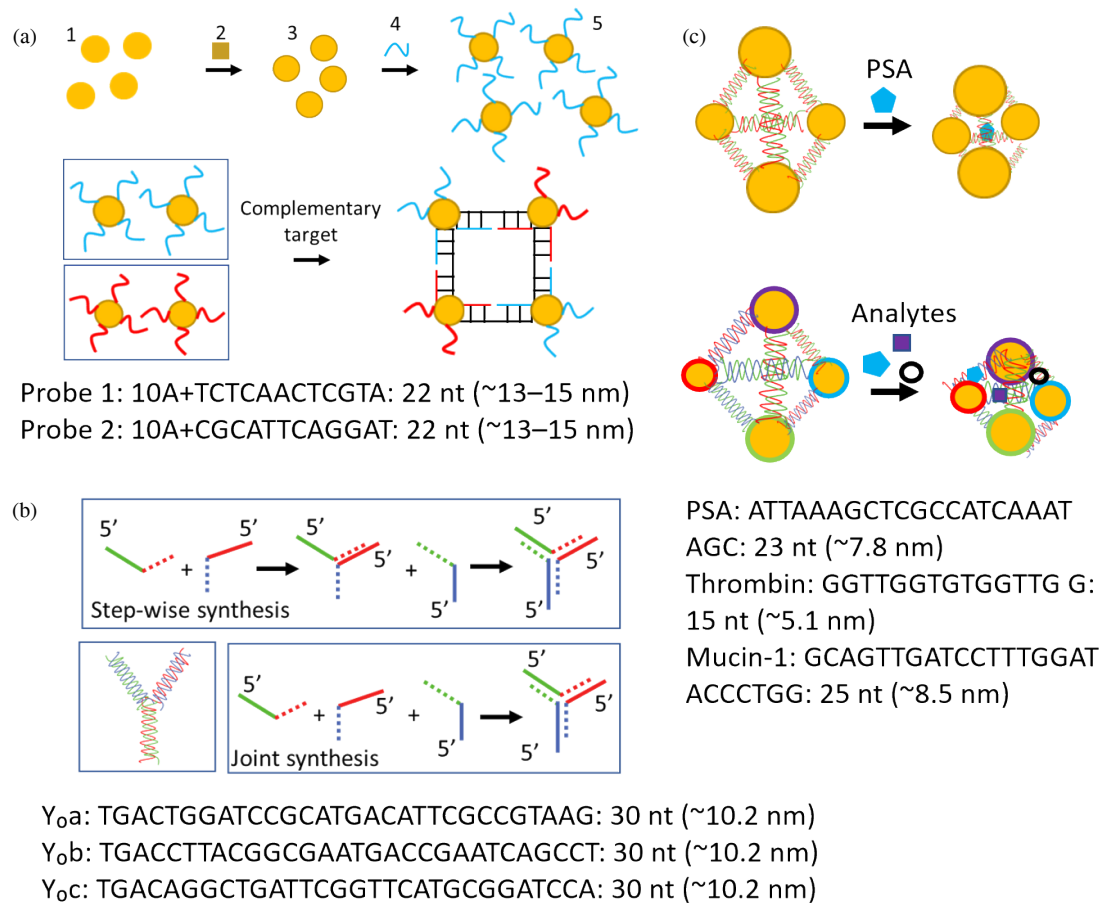


FIGURE 2. (a) Controlled aggregation of silver nanoparticles through DNA hybridization. Thiolated ssDNA sequences are immobilized on the nanoparticle surfaces. The addition of complementary linker strands induces specific base pairing, facilitating targeted nanoparticle assembly. Adapted from Ref. [78]. (b) Y-DNA design: schematic structure and sequence details [90]. (c) DNA-frame-assembled Ag pyramids for multiplexed SERS detection of PSA, thrombin, and Mucin-1. For the PSA assay, Ag NPs were functionalized with 4-ATP and PSA aptamers were incorporated into the DNA frame. For multiplexed detection, each DNA-framed Ag pyramid contained three Ag NPs, each functionalized with a distinct Raman reporter (4-ATP, NTP, or MATT), while three different aptamers, specific to PSA, thrombin, and Mucin-1, were incorporated into the three corresponding sides of the DNA frame, enabling simultaneous detection of the three biomarkers [100].

synchronized signal output in both photoelectrochemical and SERS modalities (Fig. 3(b)) [115]. Similarly, Xu's group has reported a self-assembled aptasensor based on Au dimers, where the distance between the Au nanoparticles is controlled by the conformational changes of a β -phenethylamine aptamer. In the absence of β -phenethylamine, the Au dimers were in a loose state, resulting in a weak SERS signal. However, when β -phenethylamine is present, it binds to the aptamer, causing the aptamer to fold and bring the Au nanoparticles in the dimers closer together, creating SERS hotspots (Fig. 3(c)). This enhances the SERS signal of the Raman reporter (4-MBN) located on the Au nanoparticles, allowing for the sensitive and selective detection of β -phenethylamine [116]. The same assembly logic is not restricted to optical readout. Nanoparticle probes engineered to dimerize upon target binding can be resolved as single or paired conjugates during nanopore translocation, so that bound and unbound probes are counted individually for antigen-antibody and microRNA targets without amplification or sample processing [117]. Single-entity readouts of this kind

give an orthogonal measure of the assembled fraction, which is ultimately what governs the reproducibility of assembly-driven SERS. These examples illustrate a central reconfiguration principle: aptamer folding can act as a nanoscale mechanical transducer that converts molecular binding into controlled plasmonic coupling.

Disassembly- and Release-driven Reconfiguration. Conversely, in competitive or displacement-based systems, target-induced unfolding or structural disruption of the aptamer releases a nanoparticle, causing a decrease in SERS signal [115, 118, 119]. Here, sensing relies on target-triggered deconstruction rather than assembly, demonstrating that aptamer-mediated reconfiguration can be engineered in both signal-on and signal-off formats. For example, Li's group has developed an aptamer-linked 3D DNA hydrogel for specific recognition of EpCAM in biological fluids. Upon binding to EpCAM, the aptamer triggered hydrogel disassembly, leading to the release of embedded Au@Ag nanoparticles. This structural change caused a distinct "On/Off" SERS signal and

a simultaneous “off-on” fluorescence response due to modulation of the Au@Ag-NQDs/GO system (Fig. 3(d)) [120]. Prior to this, Liang and co-workers reported a method that utilized a sandwich-like structure formed between Au@4MBA@Ag nanoparticles functionalized with a specific aptamer and Fe₃O₄@Au carrying complementary DNA. The specific binding between the aptamer and L-theanine triggers the release of Au@4MBA@Ag NPs from the Fe₃O₄@Au surface, leading to a measurable decrease in SERS signal. This release mechanism enables quantitative detection of L-theanine, with the Raman reporter (4MBA) serving as the signal output [121].

More broadly, these systems show that aptamer-based molecular recognition can be programmed to produce multiple classes of structural response, including nanoparticle assembly, disassembly, compression, unfolding, strand displacement, and reporter release. This diversity is a major advantage of aptamer-guided nanocomplexes over more static recognition formats because the signal does not depend solely on target capture, but on how target binding reorganizes the architecture of the sensing system. Therefore, these switching behaviors are highly programmable and can be engineered into hairpin structures, aptamer beacons, triplex locks, or hybridized strand-pairing networks to enable logic-gated, multi-state nanosystems.

The choice between the two formats is not arbitrary. Assembly-driven, signal-on designs suit large or multivalent targets, since each binding event adds a junction and the background is set by the unassembled state; they pay for this with slower kinetics and sensitivity to non-specific aggregation. Disassembly-driven, signal-off designs suit small molecules and competitive formats, where a single binding event is enough to break a pre-formed junction and the response is correspondingly fast; their weakness is a compressed dynamic range, and any non-specific destabilization reads as a positive. As a working rule, assembly is preferable when the target is large enough to bridge two recognition elements and the matrix is clean, and disassembly when the target is small, the required response time is short, and a ratiometric or internal-standard readout is available to separate true dissociation from drift.

2.3. Signal Transduction and Amplification Architectures

Signal transduction and amplification within aptamer-SERS platforms rely on the precise translation of molecular recognition events into nanoscale structural transformations that enhance electromagnetic (EM) field intensities. In programmable aptamer-plasmonic nanocomplexes, signal output is not a passive consequence of target capture, but the result of an engineered transduction architecture that links molecular recognition to hotspot modulation, reporter redistribution, or catalytic amplification. Central to this is the ability of aptamers to act as molecular switches, triggered by specific target binding, to modulate interparticle spacing, nanogap formation, or reporter accessibility. One prevalent strategy involves target-induced nanoparticle assembly or disassembly, discussed in Subsection 2.2, where aptamers mediate reversible and/or irreversible clustering of SERS-active nanoparticles. Upon target binding, aptamer conformational changes can reduce interparticle dis-

tances to sub-10 nm gaps, generating intense EM hotspots and sharp increases in SERS signal. In alternative formats, target binding may instead induce structural relaxation or strand displacement that disassembles aggregates, resulting in signal diminution and thereby enabling both signal-on and signal-off transduction modes.

Logic-gated and Conformation-dependent Architectures.

Logical reconfiguration of aptamers is another strategy exploited in hairpin or split-aptamer systems, where target-induced loop opening, strand hybridization, or structural unlocking alters the spatial arrangement of labeled Raman reporters. In such constructs, Raman tags are either brought into or moved out of the plasmonic hotspot region, directly modulating the SERS intensity. Aptamers are frequently integrated with molecular logic gates, where multiple input conditions (presence of two or more analytes) are required to trigger a signal output, allowing Boolean-type AND, OR, or NOT responses (Fig. 4(a)) [122, 123]. In the Hg²⁺-responsive system illustrated in Fig. 4(a), the logic output is directly coupled to a conformational transition of the DNA structure. In the absence of Hg²⁺, guanine-rich sequences can form a G-quadruplex stabilized by Hoogsteen hydrogen bonding, giving rise to a characteristic Raman band at approximately 1485 cm⁻¹. Upon Hg²⁺ recognition, formation of T-Hg²⁺-T complexes competes with and inhibits G-quadruplex formation, leading to the attenuation of this diagnostic band. Thus, the intensity change at 1485 cm⁻¹ provides a direct spectroscopic readout of the underlying DNA conformational state. This approach enhances sensing specificity in complex biological environments, reducing off-target signals and background interference. Viewed more broadly, these systems illustrate how signal transduction can be encoded at the architectural level, such that the nanosystem responds selectively to defined combinations of molecular inputs rather than to target binding alone.

Amplification-coupled Transduction Architectures.

Catalytic amplification is another robust strategy, which couples aptamer recognition with enzymatic or catalytic reactions, such as rolling circle amplification (RCA), hybridization chain reaction (HCR), or DNAzyme cascades. These reactions generate long DNA strands or secondary structures that either serve as scaffolds for assembling multiple nanoparticles or recruit large numbers of reporter molecules, significantly amplifying the SERS signal [124–126]. For example, Li's group reported the incorporation of DNA hydrogels with RCA and click chemistry for SERS-based detection of okadaic acid (OA). The hydrogel was structurally reinforced through azido-dCTP-modified RCA strands crosslinked with ethynyl-functionalized strands, creating a stable framework for sensing. Aptamers specific to OA were embedded within the hydrogel matrix to enable selective target recognition, while DNAzymes acted as catalytic amplifiers. Upon OA binding, a displacement reaction released the DNAzyme, which then cleaved substrate strands to liberate SERS-active tags (Fig. 4(b)) [124]. Similarly, Zhang's group reported a strategy for detecting bacterial lipopolysaccharides (LPS) using SERS and electrochemiluminescence. The system relies on a target-triggered RCA mechanism, which generates poly-cytosine (poly-C) DNA strands in

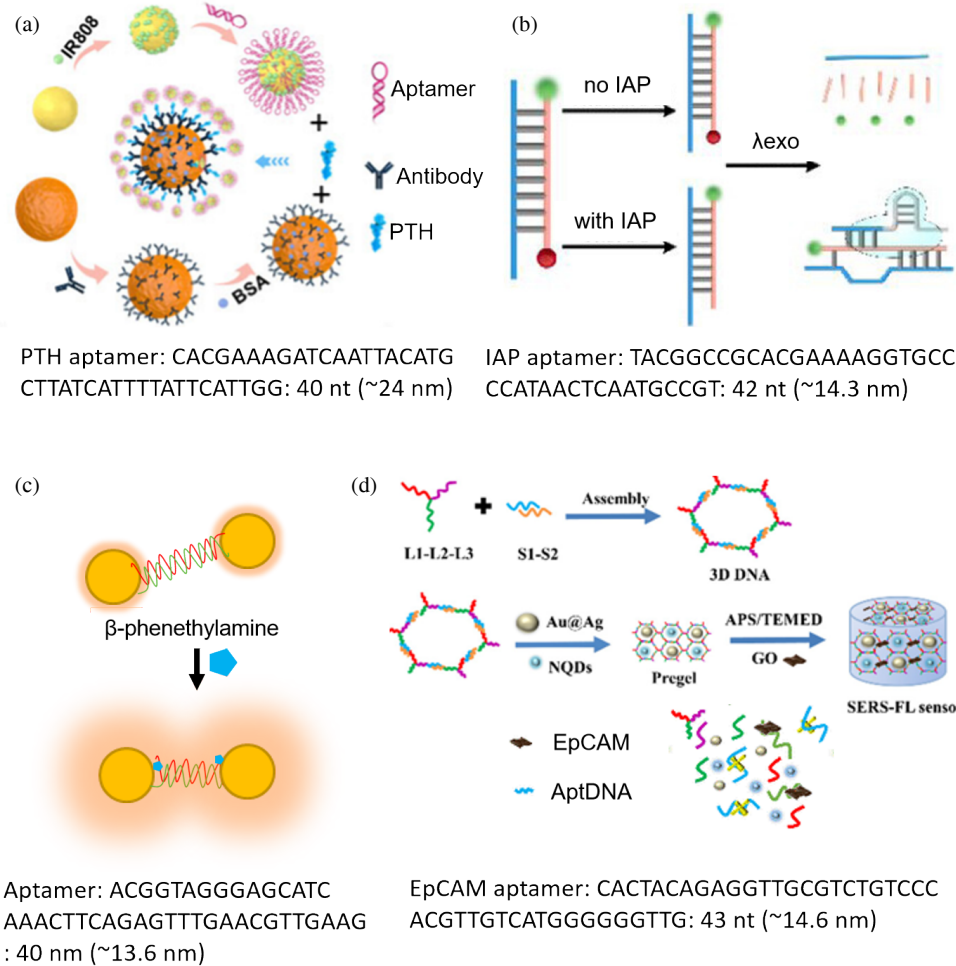


FIGURE 3. (a) Particle synthesis and PTH detection, showing SEM images of blank (control) and positive samples, along with the PTH detection procedure. Reprinted with permission from Ref. [113]. Copyright 2025 American Chemical Society. (b) Schematic of the detection principle using target-triggered CRISPR-Cas12a activity, with SERS response data for intestinal alkaline phosphatase across concentrations ($1-10^6$ pg/mL) [115]. (c) Illustration of Au dumbbell dimer structural changes in the presence/absence of β -phenethylamine [116]. (d) Preparation of 3D DNA hydrogel for dual-mode sensing, featuring EpCAM detection mechanism. Reprinted (adapted) with permission from Ref. [120]. Copyright 2024 American Chemical Society.

the presence of LPS. These poly-C strands induce anisotropic etching of Ag shells from Au@Ag nanocubes, leading to morphological changes that diminish the SERS signal [125]. Additionally, toehold-mediated strand displacement offers programmable and target-triggered nanoparticle repositioning, enabling real-time and tunable signal control. These systems are particularly important because they enable signal amplification through multi-step molecular circuitry and are well suited to multiplexed systems, where different aptamers drive distinct signal-transduction pathways on a shared nanoplatform.

Stimuli-responsive and Multimodal Transduction Architectures. These architectures have further expanded the design space of aptamer-SERS systems. In this context, aptamer-guided molecular recognition is integrated with external or endogenous triggers (such as light, pH, enzymatic activity, or redox conditions) to produce adaptive signal outputs in biologically complex environments. For example, light-controlled aptamers can regulate nanoparticle arrangement or reporter

release upon irradiation. As reported by Barman's group, a stimuli-responsive enzyme-activated nanoprobe aggregation system was developed. Gold nanocubes were coated with legumain-cleavable peptides for selective recognition of metastatic prostate cancer cells, enabling bio-orthogonal self-condensation upon internalization. In DU145 cells, high legumain activity cleaved the peptide shell, inducing nanoprobe clustering and creating dense plasmonic assemblies that enhance SERS signals [127]. In a different transduction format, Chen's group employed a target-bridged sandwich strategy for the detection of tetracycline residues. Aptamer-modified magnetic microbeads (CS@FeMMs@Apt) served as the capture probes, allowing magnetic separation and target recognition. The sensing probe consisted of 4-ATP-loaded CaCO_3 microcapsules coated with a layer-by-layer aptamer-PEI shell, which formed a complex with the target. Upon addition of EDTA, the CaCO_3 core was dissolved, releasing the 4-ATP reporter into the supernatant. The reporter generated

a “signal-on” SERS response through an AuNTs@PDMS substrate [128]. Together, these examples show that signal transduction in aptamer-SERS systems is increasingly moving beyond simple proximity readout toward adaptive, cascaded, and multimodal architectures.

In all these formats, the coupling of aptamer structural dynamics with substrate design is essential to achieving highly sensitive and selective SERS readouts. More importantly, the effectiveness of signal transduction depends on how precisely the architecture of the nanocomplex converts target recognition into a controlled optical event (Table 1). By leveraging the dual programmability of aptamer chemistry and plasmonic nanostructures, aptamer-SERS signal transduction is evolving from simple proximity-based readout to sophisticated, multi-layered architectures capable of real-time, multiplexed, and clinically relevant molecular analysis.

Overall, HCR and CHA offer the most favorable sensitivity-to-complexity balance because they provide signal amplification without requiring protein enzymes, whereas RCA offers stronger scaffold-based amplification at the cost of additional enzymatic components and workflow complexity. DNAzyme cascades provide catalytic amplification with potentially high signal gain but introduce additional sequence, substrate, and reaction-condition requirements. CRISPR-Cas12a can provide highly amplified and specific responses, but its dependence on a protein enzyme and additional reaction components increases system complexity. Thus, the optimal architecture depends on the intended application: HCR/CHA are attractive for relatively simple and scalable assays, RCA is advantageous when extensive DNA scaffolding or reporter recruitment is required, and DNAzyme or CRISPR-based architectures are more suitable when catalytic or programmable signal processing provides a specific analytical advantage.

2.4. Biomedical Functions Enabled by Programmable Aptamer-plasmonic Nanocomplexes

Programmable aptamer-plasmonic nanocomplexes have enabled transformative advances in biomedical sensing and imaging [129–132]. Rather than serving only as ultrasensitive detection platforms, these systems provide programmable analytical functions that arise from the coupling of molecular recognition, nanoscale reconfiguration, and engineered signal transduction. This section analyzes the principal biomedical functions enabled by programmable aptamer-plasmonic nanocomplexes (Table 2), including trace biomarker detection, selective profiling of complex biological targets, live-cell and extracellular vesicle analysis, and dynamic monitoring of molecular events in biologically relevant environments.

Structural Sensing of Small Molecules and Molecular Microenvironments. Small molecules such as toxins, metabolic intermediates, and pharmaceuticals are critical targets in biomedical diagnostics, food safety, and environmental monitoring [155–157]. The integration of aptamers with SERS nanostructures enables the formation of smart nanoassemblies capable of precisely responding to specific small molecules. For example, Gao’s group engineered a plasmonic dimer system using DNA origami scaffolds for the

detection of diethylstilbestrol (DES). Upon binding DES, the aptamers underwent a conformational shift, leading to dimer disassembly and a reduction in Raman intensity [158]. These examples illustrate how aptamer-guided nanocomplexes can act as molecular mechanical systems, in which small-molecule recognition is transduced through gap modulation, structural closure, or disassembly. Organic pollutants, metal ions, and pH sensing play a fundamental role in a wide range of physiological, pathological, and environmental processes. Accurate and real-time sensing of these parameters is crucial for understanding intracellular biochemical reactions, monitoring disease-related fluctuations, and conducting environmental diagnostics [159]. Aptamer-SERS nanocomplexes, particularly those incorporating structurally responsive DNA motifs, offer a highly promising approach for detecting such dynamic chemical changes with spatial and temporal resolution. The integration of functional DNA motifs such as pH-sensitive i-motifs, metal ion-specific aptamers, and DNAzymes into plasmonic nanostructures allows the transformation of molecular recognition events into measurable Raman signals [160,161]. This is achieved by translating conformational changes in DNA into spatial rearrangements of metallic nanoparticles, thereby modulating the electromagnetic field enhancement for signal amplification. Huang’s group pioneered the aptamer-ion-aptamer sandwich concept, where poly-T-modified aptamers were functionalized on a gold surface. The analyte used, PCB77, is an environmental pollutant rather than a clinical marker; the study is included here because the sandwich geometry it established underpins the metal-ion and intracellular sensors described below. Upon recognition of PCB77, the aptamers adopted an upright position to form a hairpin aptamer structure. This resulted in a change in the Raman signals of the A and G bands [162]. These systems also reveal an important design challenge: when the target is very small, such as a metal ion (e.g., Hg^{2+}), aptamer-induced structural stabilization may be weaker and must often be reinforced through sequence engineering or metal-mediated base-pairing strategies. These metal-mediated base pairs demonstrate stability comparable to Watson-Crick pairs and induce a conformational change in ssDNA, transforming it from a flexible random coil to a more rigid duplex structure [163]. Later, Wang and co-workers constructed a core-satellite SERS architecture linked by DNA tetrahedra carrying mercury-specific aptamers. Upon interaction with Hg^{2+} ions, the aptamer undergoes a conformational shift that mechanically compresses the DNA tetrahedron, drawing the satellite nanoparticles closer to the central core. This proximity intensifies the electromagnetic hotspots, leading to an enhanced SERS signal that correlates directly with Hg^{2+} concentration [164]. This molecular architecture allowed dynamic tracking of metal ion fluctuations with nanoscale precision. Another example was demonstrated where Au nanoparticles were modified with i-motif sequences. These sequences underwent structural transition depending on pH, remaining in an extended form at neutral pH but folding into a compact quadruplex under acidic conditions [165]. This reversible transition induces a change in nanoparticle proximity, dynamically regulating the SERS-aptamer complex formation.

TABLE 1. Comparison of representative DNA amplification and signal-transduction strategies across the major transduction modes discussed in Subsection 2.3. NR: not reported.

Amplification/transduction strategy	Logic-gated/conformation-dependent	Amplification-coupled transduction	Stimuli-responsive transduction	Multimodal transduction	Signal gain	Assay complexity	Enzyme requirement	Linear range	Sample matrix/validation	Replicates	Batch-to-batch variation	Specificity	Assay time
RCA	Can be integrated with target-triggered recognition	Strongly applicable	Compatible	Compatible with optical/chemical readouts	High	Moderate-high	Yes, polymerase	Study dependent	Mainly buffer; some biological matrices	NR	NR	Generally demonstrated using target/non-target controls	Typically tens of min to hours
HCR	Strongly applicable through trigger-induced hairpin opening	Strongly applicable	Compatible	Compatible	High	Moderate	No enzyme required	Study dependent	Buffer and selected biological samples	NR	NR	Target discrimination commonly reported	Typically tens of min to hours
CHA	Strongly applicable through target-triggered hairpin assembly	Strongly applicable	Compatible	Compatible	Moderate-high	Moderate	Generally enzyme-free	Study dependent	Mainly buffer; some biological matrices	NR	NR	Target discrimination commonly reported	Typically tens of min to hours
DNAzyme cascade	Can provide recognition-gated catalytic activation	Strongly applicable	Strongly applicable when activated by specific stimuli/cofactors	Strongly applicable	High	Moderate-high	Catalytic DNA, often cofactors/substrates	Study dependent	Buffer and selected biological samples	NR	NR	Often demonstrated using sequence/target controls	Typically tens of min to hours
CRISPR-Cas12a	Strongly applicable through sequence-specific target recognition	Strongly applicable through collateral cleavage	Compatible with stimulus-responsive designs	Strongly applicable	Very high	High	Cas12a enzyme	Study dependent	Buffer and increasingly biological samples	NR	NR	High sequence specificity generally demonstrated	Typically tens of min to hours

TABLE 2. Representative programmable nanoarchitectures, design mechanisms, and detection targets in aptamer-SERS biosensing. Analytical performance is reported as published. Limits of detection expressed in different units (aM or fM for nucleic acids, CFU mL⁻¹ for bacteria, cells mL⁻¹ for circulating tumor cells, particle counts for extracellular vesicles) describe different measurands and are not directly comparable across target classes; comparisons should be made within a class.

Design principle	Nanoarchitecture	Programmable mechanism	Target	Analytical performance	Ref.
Core-satellite assembly	Ag-HMS/Au-RNNP	Hybridization-driven valence control	miR-223, miR-21, miR-122	10 fM	[133]
Core-satellite assembly	Au NCs-MMPs	Sandwich hybridization	HAV-DNA strands	100 aM	[134]
Core-satellite/hierarchical assembly	Ag MNPs/Au NCs	Sandwich hybridization	miR-31, miR-21	6.49 aM, 3.46 aM	[135]
Core-satellite assembly	Au NPs	CRISPR/Cas12a amplification	Orf-cDNA	1 aM	[136]
Dimeric nanogap architecture	Janus core-shell nanogapped Au	Sandwich hybridization	miR-21	0.18 pM	[137]
DNA tetrahedron scaffold	Au-DNA tetrahedron	Aptamer-strand displacement	Telomerase, EpCAM	7.6×10^{-16} IU/0.53 pg/mL	[99]
DNA tetrahedron scaffold	Au-DNA tetrahedron/DNA-framed pyramids	Strand displacement/multiplex reconfiguration	PSA, thrombin, MUC1	–	[100]
Y-shaped DNA architecture	Au NCs	DNAzyme-mediated transduction	miR-21	2.1 pM	[138]
Y-shaped DNA architecture	Au NCs, core-satellite	DNAzyme-mediated transduction	<i>S. typhimurium</i>	4 CFU/mL	[139]
Catalytic hairpin assembly (CHA) network	Au NPs, DNA hybridization network	Toehold-mediated strand exchange	miR-652	2.91 fM	[140]
CHA network	Au NPs, core-satellite	Catalytic assembly	EpCAM	–	[141]
CHA network	Au NPs, core-satellite	Catalytic assembly	Met-T β RII, HGF, TGF β 1	–	[142]
CHA network	Au@Ag NPs, DNA duplex clusters	Catalytic assembly	CTC	8 cells/mL	[143]
Rolling circle amplification (RCA) network	Au NPs, nanoaggregates	RCA-enabled aggregation	miR-21, miR-155	0.39 fM, 0.21 fM	[144]
RCA network	Au NRs, nanoaggregates	Nanoparticle aggregation + RCA	T4 PNK	0.234 mU/mL	[145]
DNAzyme network	Au NPs, sandwich hybridization	DNAzyme + HCR amplification	miR-499	0.37 fM	[146]
DNAzyme network	Au NBPs + dsDNA, nanoaggregates	DNAzyme-mediated aggregation	miR-21	9.9 pM	[147]
DNAzyme network	Fe ₃ O ₄ @SiO ₂ @Au NPs	DNAzyme-mediated nanowalker	CTC	1 cell/mL	[148]
Hybridization chain reaction (HCR) network	Au MNPs + Au-Ag NPs, nanoaggregates	HCR amplification	<i>S. aureus</i>	0.25 CFU/mL	[149]

HCR network	Au NPs, trivalent aptamer-tetrahedral network	Branched HCR	Exosomes	1 exosome	[150]
DNA polymerase network	Au NPs, nanoaggregates + branched DNA	Polymerase-assisted amplification	E. coli O157:H7	2 CFU/mL	[151]
DNA origami/nanoarray	Au NRs, Au nanoarray sandwich	Aptamer-DNA origami assembly	CEA	–	[152]
Aptamer logic gate (Y-motif/walker)	Au NPs, ssDNA walker	Logic-enabled aptamer assembly	MUC1, EpCAM	–	[122]
DNA tweezers	Ag NPs, DNA tweezers	Strand displacement (structural reconfiguration)	AFB ₁	5.07 fg/mL	[153]
DNA tetrahedron dissociation	Au NPs/UCNPs, DNA tetrahedron	Strand displacement (nanoparticle dissociation)	Thrombin	57 aM	[98]
Dual-mode (fluorescence/SERS)	QDs + Au nanostars, core-satellite	Fluorescence quenching + SERS	MUC1	1.19 fg/mL	[154]

Abbreviations: RCA, rolling circle amplification; HCR, hybridization chain reaction; bHCR, branched hybridization chain reaction; NCs, nanocubes; NRs, nanorods; NBP, nanoprisms; UCNPs, upconversion nanoparticles; CTCs, circulating tumor cells.

This system enabled the real-time visualization of intracellular pH variations, highlighting the potential of aptamer-integrated DNA constructs in live-cell applications. Together, these systems show that programmable aptamer-plasmonic architectures are not limited to biomarker capture, but can also operate as dynamic reporters of chemical microenvironments and intracellular physicochemical states.

Dynamic Monitoring and Phenotypic Analysis in Living Systems. Monitoring of chemical and cellular events is important for understanding molecular interactions, enzyme catalysis, and cell functions. Unlike static measurements that offer a snapshot, kinetic analysis reveals how fast, efficient, and reversible these processes are. This has enabled researchers to capture transient intermediates, reaction rates, and conformational transitions in real time using SERS [166–168]. This is especially valuable in drug development, metabolic engineering, and systems biology, where time-resolved information can expose subtle regulatory mechanisms or therapeutic targets that are otherwise obscured. In this regard, Jiang's group has reported an aptamer-based tri-modal sensing platform to monitor urea with high sensitivity and selectivity in dairy products. They synthesized Fe/N-doped carbon dots (CFeN) via a hydrothermal process using ammonium ferric citrate, which efficiently catalyzed the oxidation of 3, 3', 5, 5'-tetramethylbenzidine (TMB) by H₂O₂, generating an oxidized product (TMB_{ox}) that produced distinct SERS, RRS, and fluorescence signals. The urea-aptamer suppressed the catalytic reaction, and the presence of urea restored the SERS signal by triggering a structural change in the aptamer, thus enabling real-time, signal-enhanced detection [169]. Similarly, a MOF aptasensor was reported by Li and co-workers in which ATP-aptamers and ROS responsive molecules combined with dual-mode fluorescence and SERS detection tracked ATP and ROS levels in cancer cells, related to apoptosis pathways. When ATP was present, FAM-labeled aptamers detached from the nanoprobe following incubation with cells due to the specific binding between ATP and its aptamer, re-

sulting in an enhanced fluorescence signal. Simultaneously, intracellular ROS oxidized 2-mercaptohydroquinone into 2-mercaptobenzoquinone, causing significant alterations in the SERS signal [170]. Recently, an enzyme-assisted double-cycling amplification SERS aptamer sensor was reported for the highly sensitive and specific detection of saxitoxin, a marine toxin. The system integrates the enzymes Nb.BbvCI and Klenow fragment polymerase to enable recycling of both the target and its complementary aptamer strand, enhancing signal amplification through an HCR process that forms a dendritic DNA network. This aptamer-mediated complex significantly boosts the Raman signal, achieving a detection limit as low as 1.15 pM with excellent accuracy and repeatability [171]. In summary, the integration of aptamer-driven nanostructures with SERS provides a powerful platform for real-time kinetic monitoring of biochemical reactions with high sensitivity and molecular specificity. However, challenges such as maintaining stability of the aptamer complexes under dynamic biological conditions and minimizing signal variability must be carefully addressed to ensure reliable data. Later on, building on earlier protein-level SERS profiling, Mahajan's group advanced the application of intracellular SERS nanoprobe by enabling label-free phenotyping of neuronal differentiation. Using nuclear-targeted gold nanoparticles, they achieved precise SERS readouts from the nucleus and whole-cell regions of neuroblastoma-derived progenitor and differentiated cells. Through principal component analysis of the spectral data, they clearly distinguished cell states based on molecular signatures such as increased DNA/RNA ratios and protein expression during differentiation [172]. Du et al. reported an aptamer-SERS strategy to monitor osteogenic differentiation in living bone marrow-derived stem cells (BMSCs) by targeting the key biomarker miR-144-3p. Gold nanocages (GNCs) were conjugated with aptamer hairpin DNA that specifically recognized the target miRNA and induced the assembly of GNC dimers and polymers. This platform enabled long-term, real-time tracking of miR-144-3p over a 21-day period during stem

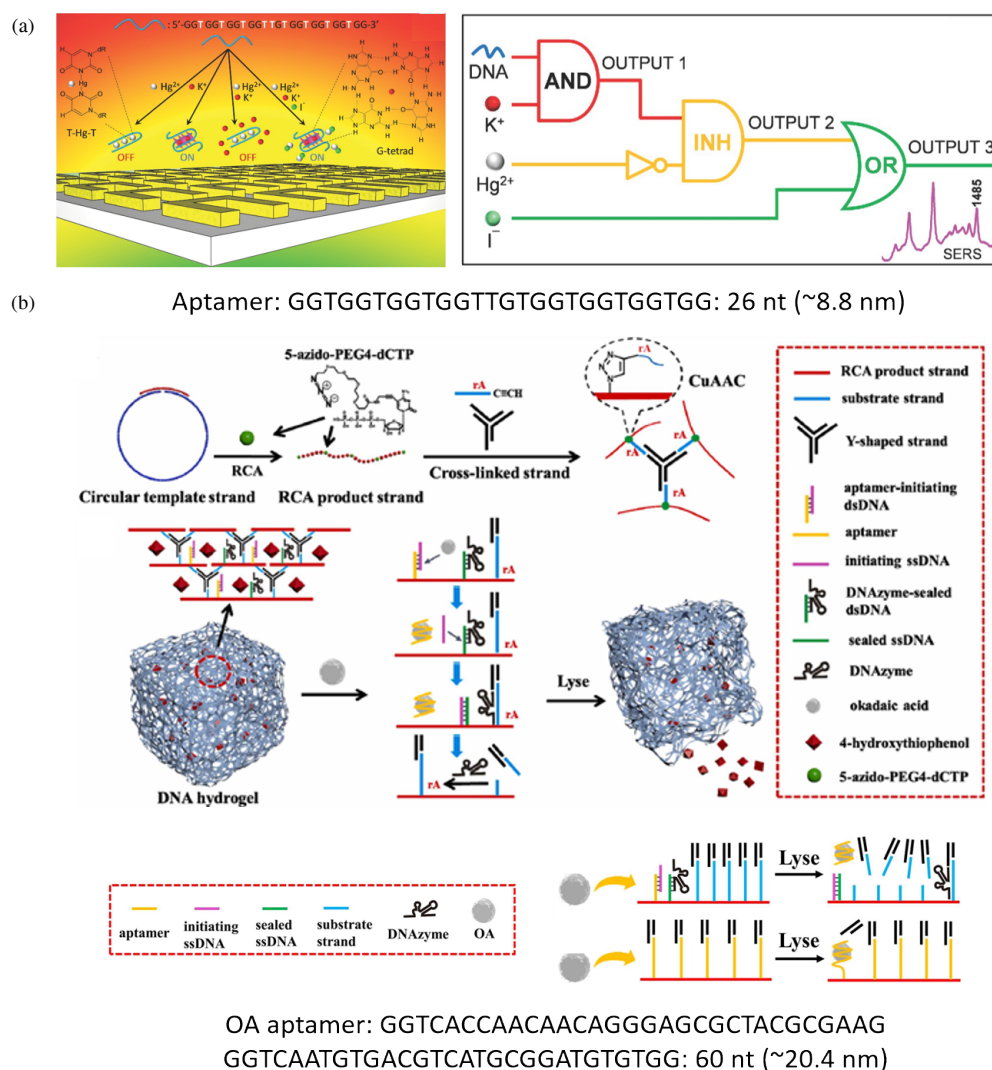


FIGURE 4. (a) Combinational logic operations (AND, INHIBIT, OR) implemented through metamaterial-enhanced SERS, where Hoogsteen hydrogen bonding activation (using GT-rich oligonucleotides with Hg^{2+} , K^+ , and I^- inputs) produces ON/OFF SERS signals at 1485 cm^{-1} (characteristic of C8 = N7-H2 bonding in G-quadruplex structures). The network map illustrates the logic gate combinations, with supporting SERS spectra showing Hg^{2+} concentration dependence. Reprinted with permission from Ref. [123]. Copyright 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) DNA hydrogel synthesis for OA detection, comparing SERS performance with/without DNase signal amplification, including concentration-dependent SERS spectra. Reprinted with permission from Ref. [124]. Copyright 2025 Elsevier B.V.

cell differentiation, achieving an impressive detection limit of 13.6 aM [173]. Importantly, this example highlights the advantages of aptamer-SERS for long-term intracellular tracking. Unlike fluorescent probes, whose signal may progressively decrease because of photobleaching, aptamer-SERS probes couple the high photostability of Raman reporters with sequence-programmed molecular recognition, enabling repeated and selective monitoring of the same biomarker over extended periods. In addition, the vibrational fingerprint readout of SERS provides richer multidimensional molecular information than conventional fluorescence channels, while the programmable recognition properties of aptamers enable selective targeting of multiple molecular markers. Thus, in the 21-day BMSC differentiation experiment, aptamer-SERS provides not only prolonged signal stability but also a framework for multiplexed

molecular and phenotypic tracking during dynamic cellular transitions.

Overall, the biomedical impact of programmable aptamer-plasmonic nanocomplexes lies in the diversity of functions they enable, such as ultrasensitive nucleic acid analysis, structurally responsive small-molecule sensing, multivalent recognition of proteins and vesicles, and dynamic monitoring of living systems. Across these use cases, their key advantage is not merely low detection limits, but the ability to translate target recognition into controlled nanoscale reconfiguration and function-specific optical output.

3. CLINICAL EVALUATION AND TRANSLATIONAL MOMENTUM OF APTAMER-SERS SYSTEMS

Aptamer-SERS systems are increasingly moving beyond proof-of-concept demonstrations toward early-stage clinical evaluation and translational validation. This transition is being driven not only by continued improvements in analytical sensitivity and multiplexing performance, but also by the growing recognition that programmable aptamer-plasmonic nanocomplexes can address clinically relevant needs in minimally invasive diagnostics, molecular stratification, and rapid point-of-care testing. Although the field remains at an emerging stage, the increasing number of registered clinical studies and translational reports indicates a measurable shift from laboratory innovation toward real-world implementation. In this context, clinical activity should be viewed not merely as evidence of application interest, but as an important indicator of translational momentum, validation readiness, and the practical maturity of aptamer-SERS technologies.

Aptamer and SERS assays have moved beyond laboratory development, with several platforms now entering clinical evaluation phases. An increasing number of SERS-enabled diagnostic platforms and aptamer-based assay formats have been registered or explored in clinically oriented settings, targeting a broad range of diseases including cancer, infectious disorders, neurological conditions, and sepsis-related biomarkers. While these technologies have shown marked progress in analytical performance, the field is still emerging, with ongoing efforts directed toward overcoming challenges in standardization, assay reproducibility, and clinical translation. Accordingly, the current landscape is best interpreted as one of translational acceleration rather than full clinical maturity. The following section outlines some representative examples of clinically registered projects and translational studies that mark this transition from benchtop innovation to real-world application.

SERS Diagnostic Assays: To assess the clinical relevance and translational momentum, we conducted a comprehensive search on ClinicalTrials.gov, which is an official registry maintained by the U.S. National Library of Medicine, and the WHO International Clinical Trials Registry Platform (ICTRP). Using the term “SERS” as an independent search query, 21 trials explicitly involving SERS-related diagnostics were identified between 2010 and 2025. Of these, 8 trials were completed and 11 were ongoing, highlighting the continued clinical interest in these platforms (Fig. 5(a)). Notably, 3 trials have progressed to advanced phases, with two in Phase 3 and one in Phase 4, underscoring their maturity and readiness for potential clinical deployment. In particular, 8 trials have been registered only in 2025. Most of the trials are related to cancer diagnostics and neurological disorders. In particular, a SERS-nanotag-based immunoassay has been reported for breast cancer evaluation (ID: NCT07018505). Similarly, a trial for early-stage diagnosis of Parkinson’s disease has been reported using SERS analysis of saliva (ID: ChiCTR2500098893). These examples suggest that SERS is increasingly being positioned not only as a high-sensitivity analytical tool, but also as a clinically relevant readout platform for accessible biofluids and disease-associated molecular signatures.

Aptamer Immunoassays: On the other hand, aptamer immunoassays have gained interest in diagnostics and drug delivery. We searched the query with the term “aptamer” and the ICTRP showed 28 registered trials between 2013–2025 (Fig. 5(b)). More broadly, this clinical activity supports the growing confidence in translationally relevant molecular technologies while underscoring the demand for ultrasensitive, rapid, and minimally invasive diagnostic tools for real-time disease monitoring across diverse clinical settings.

Importantly, we searched the clinical trial registries for studies involving SERS and aptamers jointly, with SERS serving as the detection modality and aptamers as the recognition element. However, no registered clinical trial specifically combining aptamer recognition with SERS detection was identified among studies registered between 2010 and 2025. This finding indicates that, despite the increasing clinical investigation of SERS-based diagnostics and aptamer-based technologies as independent approaches, their combination has not yet translated into a registered clinical trial, highlighting a significant translational gap. The integration of these technologies therefore remains largely at the preclinical or proof-of-concept stage. Several factors may contribute to this limited clinical translation, including the need for highly reproducible and standardized SERS substrates, robust quantitative calibration across instruments and clinical sites, validation in complex biological matrices, and prospective multicenter clinical evaluation. For aptamer-SERS systems specifically, additional challenges include demonstrating the stability and specificity of aptamer recognition under clinically relevant conditions and establishing standardized manufacturing and quality-control procedures for integrated aptamer-functionalized SERS probes.

Taken together, the available clinical and registry-based evidence indicates that SERS-based diagnostics and aptamer-based technologies are progressing independently toward clinical translation, whereas their integration into aptamer-SERS platforms remains predominantly at the preclinical and proof-of-concept stage. At the same time, the gap between promising analytical performance and clinically deployable technology remains substantial. Bridging this gap will require more than additional sensitivity gains: it will depend on rigorous validation in real patient cohorts, standardized assay design, reproducible manufacturing, and clearer alignment with regulatory expectations. Three clinical settings define where aptamer-SERS could offer something existing methods do not: severely limited sample volume, such as fine-needle aspirates or plasma below 10 μL ; multiplexed measurement of four or more markers in one reaction, where narrow vibrational bands provide encoding space that overlapping fluorophore emission cannot; and rapid decentralized testing without thermal cycling or enzymatic amplification. The quantitative case is narrower than is often claimed. A systematic comparison of thirty SERS and thirty fluorescence immunoassays places the median limit of detection one to two orders of magnitude lower for SERS (4.3×10^{-13} M against 1.5×10^{-11} M), with no significant difference in relative standard deviation between the two formats [174]. Head-to-head studies using matched reagents report similar margins: roughly fourfold on detection limit with a tenfold reduction in assay time against ELISA in one bench-

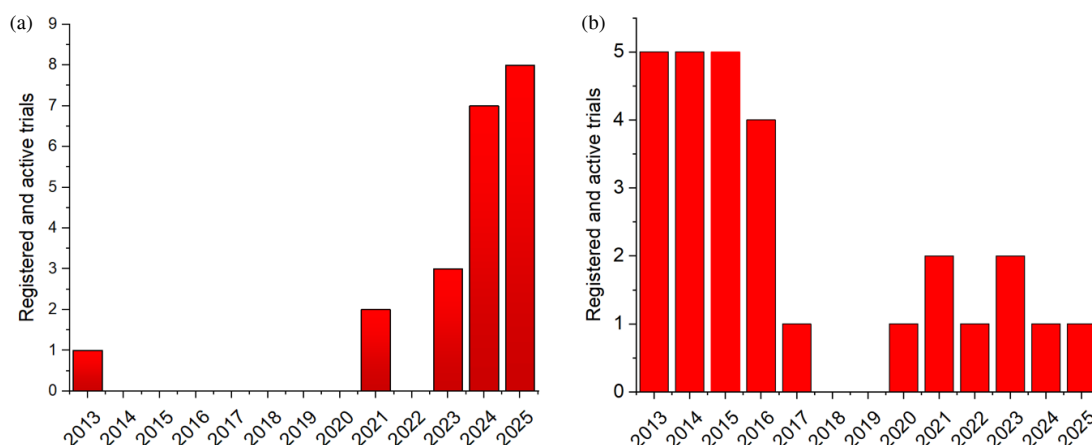


FIGURE 5. (a) Number of registered clinical trials involving SERS-based independent diagnostic approaches. (b) Number of registered clinical trials between 2013 and 2025 involving aptamer-based independent approaches. Data were obtained from ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform (ICTRP) using independent searches for SERS- and aptamer-related studies.

marked lateral-flow comparison, and roughly an order of magnitude on detection limit, 6.7- to 8-fold as measured, in a SERS-ELISA format applied to clinical samples [175]. Two caveats follow. Most of this evidence comes from antibody-based rather than aptamer-based SERS, and the largest survey covering both formats reports median detection limits of 0.5 pM for SERS immunoassays against 1.7 pM for SERS aptamer assays [175, 176]. It should be emphasized that the sensitivity of aptamer-based and antibody-based SERS assays is highly dependent on the specific target, aptamer sequence, surface chemistry, and assay format. The practical advantages of aptamers over antibodies are not primarily a matter of sensitivity, but of cost and reusability: aptamers are produced by chemical synthesis and are considerably cheaper than antibodies, and they can be stripped and regenerated for repeated use, whereas antibodies on SERS chips or nanostructures are effectively single-use. Validation cohorts also remain small, typically below one hundred participants, and interlaboratory reproducibility data are largely absent. The distinctive value of the aptamer format therefore lies in cost, regenerability, and programmable structure and switching behavior rather than in raw analytical sensitivity, which is the argument this review develops.

4. TECHNICAL CHALLENGES AND TRANSLATIONAL BARRIERS IN APTAMER-SERS BIOSENSING

Despite the rapid expansion of programmable aptamer-plasmonic nanoarchitectures in biomedical research, the transition from analytically impressive prototypes to clinically reliable systems remains constrained by a set of interconnected technical and translational barriers. These limitations do not arise from a single bottleneck, but from the cumulative effects of hotspot heterogeneity, incomplete quantitative robustness, aptamer instability in complex media, insufficient protocol standardization, and the difficulty of integrating sophisticated nanosystems into scalable and regulatory-compatible workflows. As a result, the future impact of aptamer-SERS platforms will depend not only on continued gains in sensitivity

or multiplexing, but on whether their underlying architectures can support reproducibility, manufacturability, and validation in real-world settings. To better connect nanosystem design with practical performance, the major design dimensions, failure modes, and translational constraints of programmable aptamer-plasmonic nanocomplexes are summarized in Table 3.

Among the design dimensions summarized in Table 3, signal reproducibility and quantitative robustness remain among the most immediate barriers to the practical implementation of aptamer-SERS systems.

4.1. Signal Reproducibility and Quantitative Variability in Complex Matrices

Despite this analytical potential, the application of SERS for quantitative analysis, particularly in complex biological matrices such as serum, plasma, and urine, is often hindered by substantial variability in signal reproducibility. These inconsistencies arise from a combination of substrate-related heterogeneity, sample composition, and measurement conditions, making it difficult to achieve reliable and standardized outcomes across different experiments and laboratories. For programmable aptamer-plasmonic nanocomplexes, this issue is especially critical because quantitative performance depends not only on plasmonic enhancement, but also on how consistently molecular recognition, nanoscale reconfiguration, and signal generation are coupled across different measurements and batches.

At the core of the reproducibility issue is the intrinsic nature of SERS enhancement, which is highly localized and dependent on EM hotspots formed at nanoscale gaps or surface irregularities on metallic nanostructures [109]. Because only a small fraction of the analyte molecules (typically <1%) resides within these hotspots, minor variations in particle aggregation, adsorption location, or surface chemistry can lead to large fluctuations in signal intensity. Even within a single measurement spot, small shifts in molecular orientation or positioning relative to the hotspot can produce differences of several orders of

TABLE 3. Design rules, major failure modes, and translational barriers for programmable aptamer-plasmonic nanocomplexes in biomedical SERS.

Design dimension	Common failure mode	Impact on performance	Mitigation strategy	Translational relevance	Evidence level/Ref.
Hotspot engineering and interparticle gap control	Stochastic aggregation, broad gap distribution, structural heterogeneity	Large signal variability, poor batch-to-batch reproducibility, weak quantitative reliability	DNA-guided assembly, origami, architectures, lithographic substrates, controlled conditions	Essential for standardized assays and inter-laboratory comparability	High. [177]
Aptamer orientation, density, and surface accessibility	Random immobilization, steric crowding, reduced accessibility	Lower binding efficiency, inconsistent signals, higher nonspecificity	Spacer engineering, optimized surface chemistry, controlled grafting density, antifouling layers	Critical for robustness in real biological matrices	Moderate. [178, 179]
Aptamer structural stability	Nuclease degradation, conformational instability, loss of binding in complex media	Reduced sensitivity, poor reproducibility, limited shelf life	Chemical modification, terminal protection, LNA incorporation, optimized storage buffers	Necessary for clinical deployment and product stability	Moderate. [180]
Target-responsive reconfiguration fidelity	Partial folding, off-target switching, incomplete assembly/disassembly	Ambiguous signal output, reduced specificity, unstable dynamic range	Sequence optimization, truncation/mutagenesis, structure-guided design, validation of switching behavior	Important for reliable molecular-state discrimination	Emerging. [180]
Reporter positioning and signal-output control	Variable reporter loading, non-uniform reporter distance, reporter leakage	Signal drift, poor comparability across assays, reduced sensitivity	Ratiometric design, encoded reporter placement, internal standards, covalent immobilization strategies	Supports quantitative and multiplexed assay performance	Moderate. [181]
Matrix compatibility and nonspecific adsorption	Protein fouling, nonspecific adsorption, salt-induced aggregation, background interference	False positives/negatives, loss of selectivity, distorted spectra	PEGylation, BSA/passivation layers, buffer optimization, magnetic separation, microfluidic preprocessing	Central to clinical sample analysis and real-world usability	High. [182]
Signal transduction architecture	Weak coupling between binding and signal generation, incomplete cascade activation, transduction leakage	Low gain, poor sensitivity, unstable response curves	Mechanistically validated architectures, cascade optimization, logic-gated control, integrated amplification modules	Determines whether performance can scale beyond proof-of-concept	Moderate. [183]
Quantitative calibration and normalization	Dependence on absolute signal intensity, instrument-to-instrument variability, local hotspot dependence	Limited quantification accuracy, poor reproducibility across platforms	Internal references and standards, ratiometric analysis, digital SERS approaches, calibration models	Required for regulatory acceptance and clinical interpretation	High. [184]

Multiplexing capability	Spectral overlap, cross-reactivity, reporter crosstalk, unequal amplification efficiency	Reduced accuracy in multi-analyte detection	Carefully selected reporters, logic-gated architectures, spectral deconvolution, machine-learning-assisted classification	Important for high-content diagnostics and biomarker panels	Moderate. [185]
Manufacturability and scale-up	Complex multistep synthesis, poor process control, low yield, unstable products	Limited scalability, high production cost, poor lot consistency	Simplified synthesis routes, modular assembly strategies, GMP-compatible workflows, process standardization	Major bottleneck for translation from lab prototypes to products	Emerging. [186]
Standardization and inter-laboratory reproducibility	Laboratory-specific substrates, different laser conditions, inconsistent preprocessing pipelines	Poor comparability of published results, low confidence in benchmarking	SOPs, common reference materials, harmonized reporting standards, interlaboratory validation studies	Essential for field-wide maturity and guideline development	High. [187, 188]
Regulatory and clinical validation	Insufficient validation cohorts, unclear performance benchmarks, lack of regulatory pathways for aptamer-SERS systems	Delayed translation, difficulty in regulatory review, weak clinical adoption	Prospective validation, multicenter studies, toxicology assessment, clinically relevant benchmarking, early regulatory alignment	Determines transition from experimental platform to approved diagnostic technology	High for the need/barrier, but emerging for actual aptamer-SERS implementation. [189]
Integration with AI, microfluidics, and portable devices	Overfitted models, poor external validation, unstable device integration, loss of assay performance outside lab settings	Reduced trustworthiness, poor field performance, limited adoption in decentralized settings	External validation datasets, interpretable ML workflows, integrated fluidic control, portable Raman optimization	Key for next-generation point-of-care and decentralized diagnostics	Moderate. [190]
Overall cost, scalability, and shelf-life	Expensive synthesis, lot-to-lot variation, activity loss during storage	High barrier to commercialization, short product lifespan	Streamlined GMP synthesis, lyophilization, accelerated stability studies	Directly determines commercial viability and supply chain feasibility	Emerging. [191]

magnitude in Raman signal, particularly when analyte concentrations approach the single-molecule level. As a result, analytical sensitivity and quantitative reliability do not necessarily scale together: systems capable of extreme enhancement may still suffer from poor measurement-to-measurement consistency.

In addition to spatial heterogeneity, other variables further complicate quantification, such as inconsistencies in SERS substrate fabrication, uneven laser illumination profiles, differences in laser power or exposure time, and fluctuations in environmental conditions such as temperature or humidity. Sample preparation protocols, such as dilution, filtration, and incuba-

tion steps, also contribute to variability, especially when dealing with complex matrices where protein fouling or nonspecific adsorption can mask or distort the true signal. In aptamer-SERS systems, these effects are often amplified by variations in aptamer surface density, orientation, conformational accessibility, and target-induced structural response, all of which directly influence hotspot formation and reporter behavior.

Therefore, efforts are increasingly focused on improving substrate design and functionalization strategies [192]. Techniques such as lithographically patterned surfaces, self-assembled monolayers, and template-assisted nanoparticle arrays are being developed to create more uniform and re-

producible enhancement regions. In parallel, tailored surface chemistries are being explored to optimize analyte-surface interactions, enhance selectivity, and reduce nonspecific adsorption. For aptamer-guided nanocomplexes, additional emphasis must be placed on encoded control of interparticle spacing, reporter placement, and aptamer presentation, since these parameters determine whether signal generation remains structurally reproducible under biologically relevant conditions. These developments, combined with rigorous calibration and validation protocols, represent important steps toward realizing the full potential of SERS as a quantitative biosensing tool in biomedical research.

Beyond substrate engineering alone, quantitative robustness will likely depend on the routine integration of internal standards, ratiometric readouts, digital SERS strategies, and harmonized preprocessing workflows. In this sense, reproducibility should be treated not merely as an analytical limitation to be minimized, but as a primary design criterion for clinically relevant aptamer-SERS platforms.

4.2. Aptamer Stability, Target Affinity, and Structural Robustness

Another key design dimension highlighted in Table 3 concerns whether aptamers retain both molecular integrity and functional responsiveness under biologically relevant conditions. As nucleic acid-based recognition elements, aptamers are inherently susceptible to chemical and enzymatic (nuclease) degradation in biologically complex environments such as serum, plasma, and cell culture media [193]. For programmable aptamer-SERS systems, this limitation is particularly significant because aptamers do not act solely as binding ligands: they also define nanocomplex assembly, structural reconfiguration, and signal-output behavior. Consequently, any loss of aptamer integrity can affect not only target recognition, but also hotspot geometry, reporter positioning, and overall analytical reliability.

Chemical and Structural Stabilization Strategies. Improving the structural resilience and target-recognition capability of aptamers is therefore a central requirement for translating these systems beyond proof-of-concept studies. Several molecular design strategies have been developed to enhance aptamer stability against nuclease degradation and environmental stress. For example, Levy's group investigated the effects of chemical modifications such as phosphorothioate backbones, 2'-hydroxyl, 2'-O-methyl, and 2'-fluoro substitutions following exposure to frozen and fresh human serum as well as mouse serum [194]. Contrary to expectation, 2'-fluoro RNA proved only about as stable as unmodified DNA, and a 3' inverted deoxythymidine cap gave at most a modest gain. The longest serum half-lives came from fully modified oligonucleotides, either uniformly 2'-O-methyl or 2'-O-methyl A, C and U combined with 2'-fluoro G, leading the authors to argue that in vivo use requires full rather than partial modification. In parallel, several studies have reported the incorporation of LNAs to improve nuclease resistance, particularly in serum-containing environments [195, 196]. Terminal modifications such as 5' PEGylation extend circulation time and improve solubility, although the contribution of 3' capping alone to serum stability

now appears smaller than once assumed [197]. More robust structural variants, such as L-form aptamers (Spiegelmers) and circular/dumbbell-shaped aptamer designs offer greater conformational rigidity and enhanced resistance to enzymatic attack [198]. For long-term preservation, aptamers are often freeze-dried in the presence of stabilizers such as trehalose and stored in buffered solutions, typically at -20°C or below, to minimize loss of activity and degradation resulting from repeated freeze-thaw cycles. As such, these strategies show that aptamer stabilization is not merely a matter of molecular preservation, but a prerequisite for maintaining predictable nanosystem behavior under practical assay conditions.

Affinity Retention and Functional Robustness in Complex Media. At the same time, analytical performance depends not only on aptamer survival, but also on the retention of strong and specific target binding under operational conditions. Techniques applied after the initial SELEX process, such as sequence truncation and site-directed mutagenesis, can refine aptamer structure and improve both binding strength and selectivity. In the context of aptamer-SERS nanocomplexes, however, affinity must be considered together with structural function: an aptamer that binds strongly but folds unpredictably, or that performs inconsistently when surface-immobilized, may still compromise signal reproducibility and transduction efficiency. The use of multivalent formats, in which multiple aptamer units are incorporated into a single construct, can significantly increase avidity, especially when targeting large biomolecular assemblies such as extracellular vesicles or bacterial surfaces. Signal amplification mechanisms, including rolling circle amplification, hybridization chain reaction, and DNAzyme-mediated amplification, have also been shown to reduce detection thresholds substantially [199–202]. In the context of SERS, incorporating strong Raman reporters or adopting sandwich-style detection schemes can further enhance analytical sensitivity.

One of the critical hurdles in analyzing biological fluids is the occurrence of nonspecific adsorption, which can obscure true signals and reduce apparent binding fidelity. This challenge can be addressed using molecular spacers such as poly-A or poly-T linkers, flexible surface-binding chemistries, and passivation agents including BSA and PEG. Moreover, maintaining appropriate buffer conditions, especially with respect to pH and ionic strength, is vital for preserving the tertiary structure of aptamers, particularly those that form G-quadruplex or stem-loop motifs. For clinically relevant aptamer-SERS platforms, stability, affinity, and structural robustness must be optimized together (Table 4). Several chemical and structural modification strategies can improve aptamer stability in biological environments, while differing in their effects on affinity, switching behavior, and manufacturing scalability, including production cost¹.

¹Relative cost categories are qualitative and depend strongly on sequence length, synthesis scale, purification, modification density, supplier, and intended grade. Manufacturing scalability is provided as practical engineering guidance rather than universal prices.

TABLE 4. Comparison of aptamer stabilization strategies in terms of serum stability, effects on affinity and switching behavior, and relative manufacturing complexity.

Strategy	Principle	Serum stability	Effect on affinity/switching	Scalability/manufacturing
Terminal modification (3'-inverted dT, PEG)	Exonuclease protection	Modest improvement	Usually minimal	High
Phosphorothioate backbone	Nuclease resistance	Moderate-high	May alter binding	High
LNA modification	Increased rigidity and stability	High	Can improve or impair switching	Moderate
Spiegelmer (L-oligonucleotide)	Complete nuclease resistance	Very high	Requires mirror-image target selection	Lower
Circular aptamer	Eliminates free ends	High	May constrain conformational switching	Moderate

4.3. Lack of Protocol Standardization and Interlaboratory Comparability

Beyond molecular robustness at the aptamer level, the practical advancement of aptamer-SERS systems also depends on whether assay performance can be reproduced across laboratories and measurement workflows. Another major barrier to the broader implementation of aptamer-SERS biosensing is the lack of standardized protocols for substrate fabrication, assay execution, spectral acquisition, and data processing. Even when individual studies report excellent sensitivity, selectivity, or multiplexing performance, the absence of harmonized methodological frameworks makes it difficult to compare results across laboratories, benchmark architectures reliably, or assess whether analytical performance is genuinely reproducible. Recent collaborative studies have attempted to address this challenge [203].

Interlaboratory Reproducibility and Benchmarking Constraints. A large-scale European interlaboratory study investigated whether a SERS method for detecting a known analyte in buffered aqueous solution could produce consistent results across multiple research centers. While reasonable reproducibility was observed for well-defined systems and protocols supported by detailed standard operating procedures (SOPs), the findings confirmed that reproducibility remains a limiting factor for broader regulatory adoption. Even under controlled conditions, SERS performance varied due to differences in substrate design, laser wavelength selection, and data preprocessing protocols. These findings are particularly relevant to aptamer-SERS systems, where additional layers of variability arise from aptamer immobilization, target-responsive reconfiguration, and reporter placement, all of which may differ subtly across platforms and laboratories. This suggests that establishing universal guidelines and SOPs is an urgent requirement for reliable quantification. The use of internal standards and statistical normalization has shown promise in mitigating variability. Approaches such as ratiometric analysis, digital SERS (which

involves counting discrete SERS events), and the use of isotopically labeled molecules or reference dyes help reduce dependence on absolute signal intensity [192, 204]. Moreover, the application of chemometric tools, including principal component analysis and machine-learning algorithms, has enabled improved signal interpretation, especially in multiplexed or noisy biological settings. However, these analytical tools can improve comparability only when they are embedded within standardized workflows for sample preparation, spectral acquisition, preprocessing, and data reporting.

Standardization Needs in Real Biological Matrices. Despite progress, reproducibility challenges are amplified in real biological samples, where the matrix itself introduces variability. Biological fluids contain diverse proteins, ions, lipids, and small molecules that can interfere with analyte binding, affect nanoparticle aggregation, or generate background signals. This is particularly problematic for aptamer-SERS biosensing platforms, where aptamer orientation, density, and surface accessibility play critical roles in determining binding efficiency and consequently SERS output [205]. As a result, field-wide maturity will depend not only on better-performing nanosystems, but also on whether the community converges on shared validation practices, reference conditions, and reporting standards. Without this level of standardization, even highly sophisticated programmable nanocomplexes may remain difficult to reproduce, benchmark, and translate beyond isolated proof-of-concept studies.

4.4. Regulatory and Translational Constraints

The clinical translation of aptamer-SERS immunoassays is currently constrained not only by technical limitations, but also by the lack of clear regulatory and validation pathways. Unlike conventional antibody-based immunoassays, such as ELISA and Western blot, aptamer-based diagnostics remain outside mainstream regulatory frameworks due to their synthetic nature, structural variability, and relatively short commercial his-

tory. Moreover, SERS detection lacks standardized criteria for defining signal thresholds, quantification accuracy, and reproducibility across instruments, substrates, and data-processing workflows. These uncertainties complicate the demonstration of analytical validity, clinical reliability, and comparability under regulatory evaluation frameworks, including technology readiness level (TRL) frameworks and guidance documents for in vitro diagnostic test validation such as WHO's TGS-4 Guidance for Test Method Validation [206, 207].

Manufacturing, Validation, and Safety Constraints. Even when performance metrics such as sensitivity or dynamic range surpass those of conventional methods, important barriers remain at the translational interface. For instance, many aptamer-SERS assays rely on complex and costly nanoparticle synthesis, sophisticated surface functionalization, or architecture-specific assembly routes that are difficult to reproduce at industrial scale under GMP conditions. Furthermore, regulatory authorities often require detailed toxicological profiles of nanomaterials, especially for any in vivo diagnostic use, yet harmonized safety evaluation protocols for SERS-active nanoparticles are still lacking. As a result, systems that are analytically impressive in academic settings may still fall short when evaluated against manufacturability, quality control, safety, and lot-to-lot consistency requirements.

Commercialization and Regulatory Alignment. In addition, aptamers themselves often face challenges in intellectual property protection, with overlapping patents on SELEX variants, sequence libraries, and modification strategies, making licensing and commercialization more complex. Many commercial ventures therefore remain positioned between successful proof-of-concept and clinically approved product, due to fragmented regulatory expectations, unclear classification frameworks, and insufficient clinical validation. This translational gap is particularly important for programmable aptamer-plasmonic nanocomplexes, whose performance often depends on coupled variables, such as aptamer presentation, reporter positioning, hotspot geometry, and dynamic reconfiguration, that are not yet captured by conventional validation paradigms.

To bridge this gap, there is a critical need for multidisciplinary and inter-institutional collaborations focused on validation frameworks, the establishment of standard operating procedures, harmonization of data formats, and the standardization of SERS substrates, aptamer formats, and reporting practices. More broadly, the field will require earlier alignment between nanosystem design, clinical benchmarking, manufacturing feasibility, and regulatory expectations. Without this alignment, even highly sophisticated aptamer-SERS platforms may struggle to progress from promising analytical technologies to clinically deployable diagnostic systems. These constraints define the conditions under which next-generation aptamer-SERS systems must now be engineered, validated, and integrated into clinically meaningful workflows.

5. FUTURE PERSPECTIVES AND CONCLUSIONS

Aptamer-SERS biosensing is now entering a decisive stage of development, in which future progress will depend less on achieving isolated records in analytical sensitivity and more

on whether programmable nanosystems can be engineered for robustness, interoperability, and clinical relevance. The convergence of aptamer programmability, plasmonic nanoengineering, machine-learning-assisted analysis, and portable diagnostic hardware creates a compelling opportunity to move the field from analytically impressive prototypes toward deployable biomedical technologies. In this context, the most important future directions are not independent trends, but interconnected requirements for translation: quantitative robustness, automated data interpretation, workflow integration, and compatibility with decentralized testing environments.

5.1. AI-integrated Aptamer-SERS Systems for Robust Analysis

Advances in artificial intelligence (AI) have significantly expanded the utility of SERS and aptamer-based immunoassays, particularly by improving the interpretation of complex, variable, and high-dimensional spectral datasets [208–210]. When combined with optical sensor arrays and microfluidic platforms, machine-learning-assisted SERS systems are capable of high-throughput, low-volume, and multiplexed analyses that are highly attractive for point-of-care diagnostics. These systems demonstrate efficiency, fast responsiveness, and operational simplicity [211, 212]. The fusion of SERS, microfluidics, and data-driven analysis not only streamlines sample handling but also improves spectral classification and pattern recognition, which is especially critical in the detection of viral infections and other clinically heterogeneous conditions where speed, accuracy, and reproducibility are paramount [213]. Chemometric and machine-learning-driven analytical frameworks are helping reposition SERS from a specialized detection method to a more adaptable clinical tool for infectious disease surveillance and bioassay development [214]. SERS-based immunoassays enhanced by intelligent algorithms are now being used to detect intermittent disease markers and identify previously elusive sources of infection. Such capabilities could significantly improve disease prevention strategies and therapeutic monitoring [215]. For instance, exosomes released by tumor cells carry valuable biochemical information that can be analyzed using aptamer nanotags integrated with microfluidic-SERS platforms. When integrated with machine-learning workflows, these vesicles offer a pathway toward non-invasive, liquid-biopsy-based cancer diagnostics and cellular phenotyping (Fig. 6(a)) [216, 217].

Applications in live-cell SERS imaging are also gaining ground, enabling real-time molecular probing of organelles, chemical gradients, and intracellular interactions. In these contexts, AI is valuable not only for classification, but also for managing spectral complexity, denoising, feature extraction, and pattern recognition in data-rich biological environments [218]. In particular, deep learning algorithms such as RNN and CNN have shown strong potential for interpreting SERS spectra from large datasets [219, 220]. These models have been applied to differentiate antibiotic-resistant pathogens, diagnose hemorrhagic fever viruses, and assess cardiovascular risk based on serum profiles (Fig. 6(b)) [220–222]. Single-vesicle SERS measurements, when combined with machine-learning processing, have also facilitated the stratification of glioblastoma

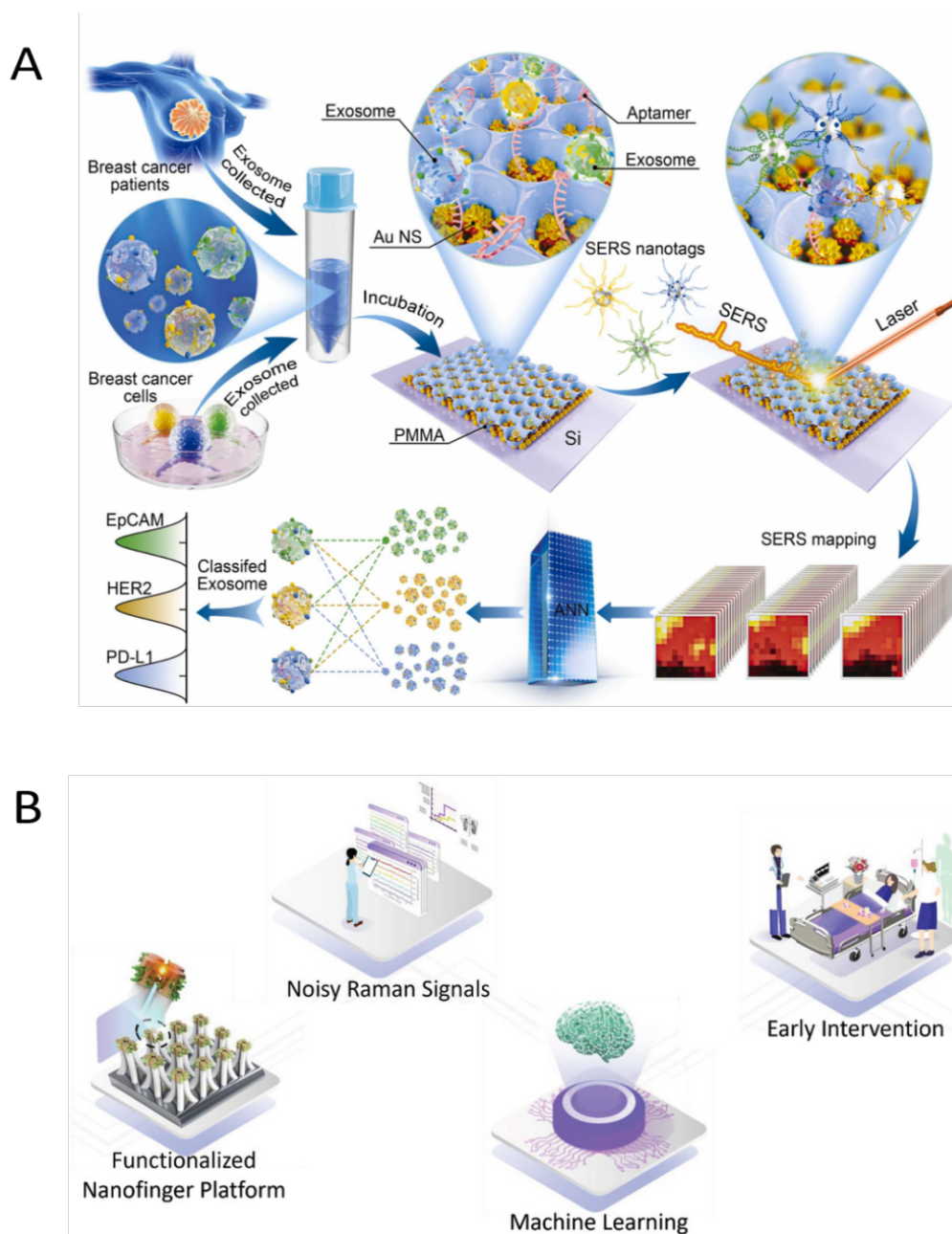
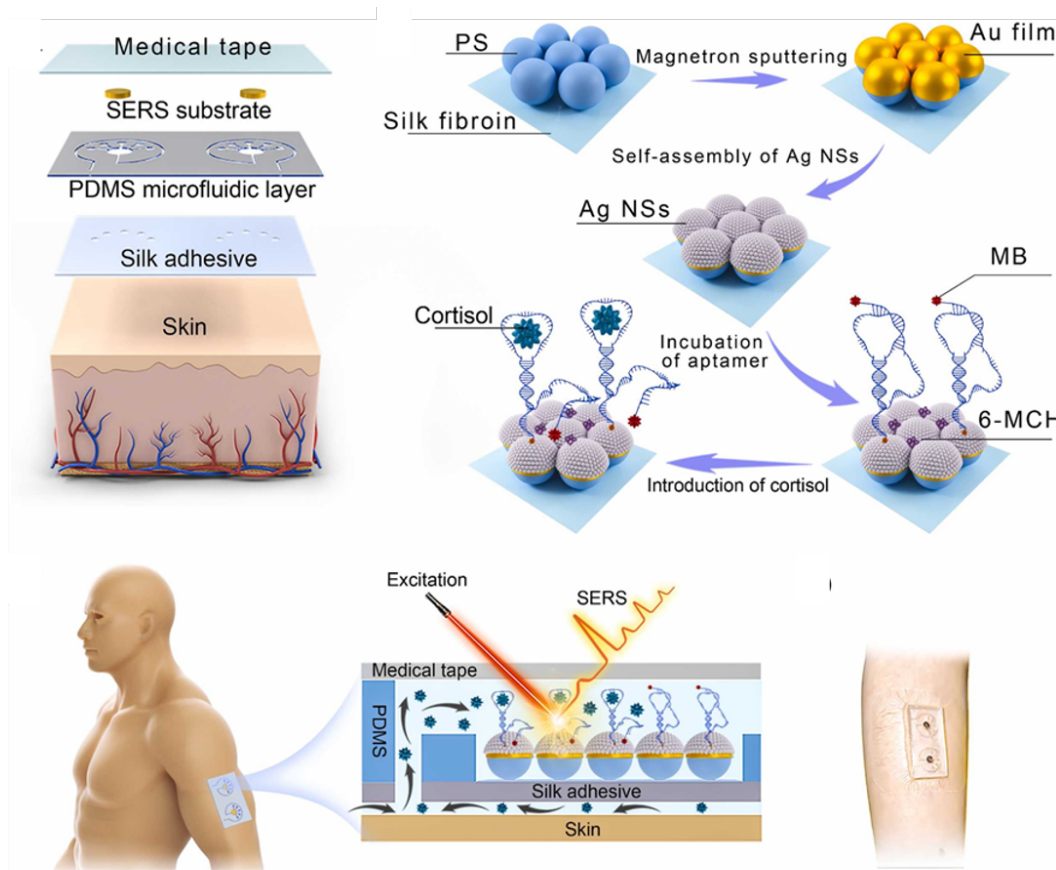


FIGURE 6. AI-integrated SERS platforms for cancer subtyping and cardiovascular diagnostics. (A) (a) Workflow of the plasmonic nanoarray chip for single-exosome analysis and breast cancer classification, featuring: (i) Schematic of the two-layer CNN architecture for exosome/blood sample classification; (ii-iii) Training performance metrics ($n = 1,361$ exosomes) showing confusion matrix and ROC curves; (iv-v) Validation results ($n = 454$ exosomes) with corresponding classification metrics; (vi-vii) Clinical discrimination of healthy controls ($n = 2$) versus three breast cancer subtypes ($n = 6$ patients). Reprinted with permission from Ref. [227]. Copyright 2025 Elsevier B.V. (B) Collapsed nanofinger SERS platform for acute myocardial infarction detection: Comparative performance of machine learning models including (a) human experts, (b) Decision Tree, (c) SVM, (d) KNN, (e) Random Forest, and (f) ensemble voting classifier, presented as normalized confusion matrices. Reprinted with permission from Ref. [222].

patients during liquid biopsy evaluations [223]. Moreover, spectral signatures of stroke-specific metabolites have been harnessed by machine-learning models for rapid and accurate screening, supporting the vision of data-driven early diagnosis [224].

Machine learning enters aptamer-SERS at three distinct points, only one of which is spectral. Sequence-level models

generate and rank candidate aptamers directly from selection data, identify high-affinity binders in early unenriched SELEX rounds, and in reported cases reduce the number of selection rounds required by up to 80% while improving affinity over the best experimentally selected candidates [225–228]. Structure-level models predict folding and switching thermodynamics, the layer that matters most here, because a target-responsive



Cortisol aptamer: CTC TCG GGA CGA CGC CAG AAG TTT ACG AGG
 ATA TGG TAA CAT AGT CGT CCC TTT TTT TTT TTT GGT CTT CA: 71 nt
 (~24.1 nm)

FIGURE 7. Silk fibroin-based wearable SERS biosensor: (i) Fabrication process of SFOS/Au/Ag nanoshell substrate. (ii) Cortisol detection mechanism in sweat. (iii) Exploded view of the microfluidic patch design. (iv) Operational demonstration on forearm with inset showing cross-sectional architecture. Adapted from Ref. [235].

linker has to be designed to change conformation rather than merely to bind [229,230]. Only the third point, spectral classification and denoising, is shared with SERS generally. Separating these three is what turns a general argument for machine learning into an aptamer-specific one.

Nevertheless, the translational value of AI-integrated aptamer-SERS systems will depend on more than classification accuracy alone. Future progress will require externally validated datasets, interpretable analytical pipelines, standardized preprocessing workflows, and careful control of bias introduced by limited training cohorts or laboratory-specific acquisition conditions. In this sense, AI should not be viewed as an optional add-on, but as a tool that must be integrated with nanosystem design and assay standardization to improve quantitative robustness and clinical trustworthiness.

5.2. Smart Point-of-Care Systems and Deployable Platforms

In the post-COVID era, the development of smart POC biosensing platforms has accelerated markedly, driven by the demand for compact, user-friendly, and high-performance diagnostics beyond centralized laboratories. This shift has been catalyzed by the urgent need for rapid, reliable, and field-deployable tools. Among the most promising approaches, aptamer-SERS-based immunoassays have emerged as a powerful alternative to traditional antibody-based tests [231]. Their programmability, high specificity, and chemical stability make them well suited for integration into next-generation diagnostic devices and microsystems [232]. Advanced lateral flow systems leveraging SERS readouts [233], microfluidic devices designed for high-throughput phenotyping [234], wearable SERS systems (Fig. 7) [235], and digital approaches for COVID-19 detection [236,237] are reshaping the diagnostic landscape with capabilities once limited to benchtop instruments. Among these approaches, microfluidic integration with aptamer-SERS assays enables the simultaneous detection of multiple biomarkers

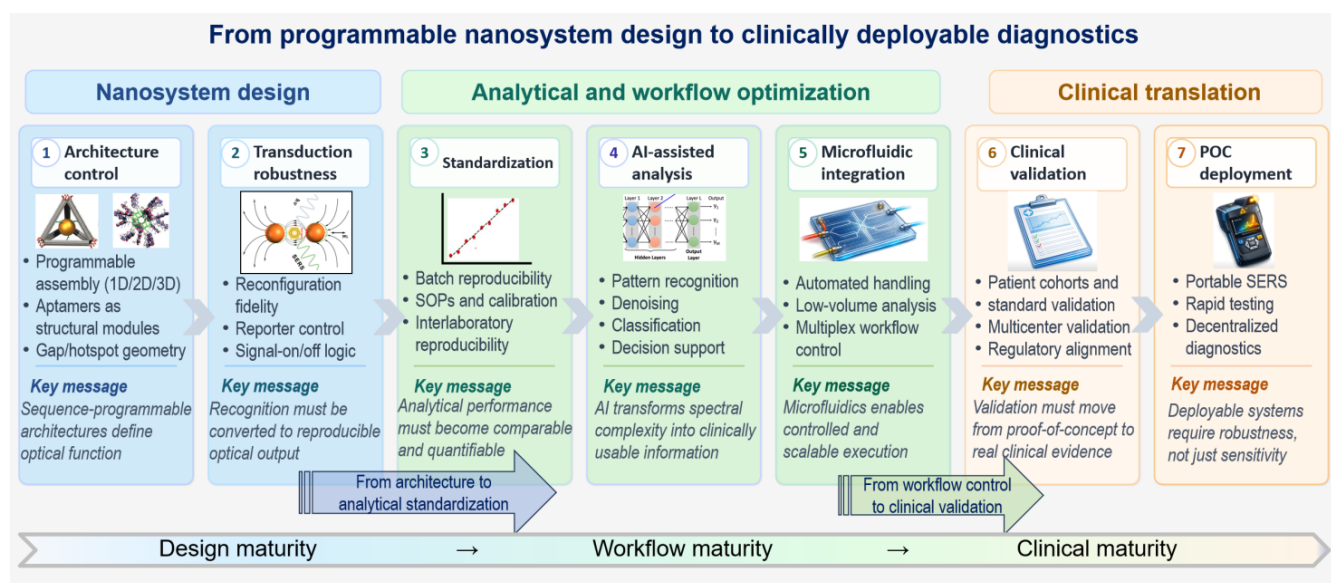


FIGURE 8. Translational roadmap for programmable aptamer-plasmonic nanocomplexes in biomedical SERS. The roadmap summarizes the interconnected design, analytical, and validation steps required to move aptamer-SERS systems from proof-of-concept nanosystems to clinically deployable diagnostic platforms. Translation depends on coordinated progress in architecture control, transduction robustness, standardization, AI-assisted spectral analysis, microfluidic integration, clinical validation, and point-of-care deployment. Rather than representing independent innovation tracks, these dimensions act as sequential and interdependent requirements for achieving reproducible, interoperable, and clinically relevant aptamer-SERS technologies.

within a single analytical platform [238, 239]. By immobilizing distinct aptamers in spatially separated microchannels or reaction zones, multiple disease-related targets can be captured and identified in parallel. This approach enhances diagnostic precision by reflecting the complex biomarker profiles associated with real physiological or pathological states. The microfluidic system ensures controlled fluid handling, reduced sample consumption, and improved assay reproducibility. Moreover, multiplexed SERS readouts provide rich spectral information that can be rapidly analyzed for quantitative comparison across targets.

However, the practical success of smart POC aptamer-SERS systems will depend on more than miniaturization alone. Future platforms must combine robust nanocomplex design with simplified sample preparation, stable signal calibration, automated spectral interpretation, and device-level reproducibility. For real-world deployment, portability must be accompanied by operational reliability, manufacturing consistency, and compatibility with clinical workflows outside highly controlled laboratory settings. In this regard, the most promising platforms will likely be those that integrate programmable aptamer-plasmonic architectures with microfluidic automation, internal referencing strategies, and user-independent decision support. Rather than representing separate innovation tracks, these advances define an integrated translational roadmap for programmable aptamer-plasmonic nanocomplexes (Fig. 8).

5.3. Outlook and Concluding Remarks

Looking ahead, the evolution of aptamer-SERS biosensing holds immense promise for transforming molecular diagnostics. Yet the long-term impact of the field will depend on

whether programmable aptamer-plasmonic nanocomplexes can move beyond isolated analytical excellence and toward reproducible, validated, and interoperable sensing systems. The convergence of programmable nanostructures, microfluidic integration, portable Raman platforms, and machine-learning-assisted analysis is paving the way for smart diagnostic systems capable of sensitive, specific, and high-content molecular interrogation. What is now needed is a shift in emphasis from demonstrating what aptamer-SERS systems can detect to engineering how reliably, comparably, and deployable they can operate in real biomedical settings.

Three design principles survive the evidence reviewed here. First, deterministic control of the gap matters more than maximizing enhancement, because the limiting variable in reported translational failures is the width of the signal distribution rather than its mean. Second, Raman reporters should be positioned rather than adsorbed; embedded reporters combined with an internal standard convert an arbitrary intensity into a ratio that can be compared across batches and instruments. Third, recognition and geometry should be decoupled, with the aptamer carrying molecular recognition and a rigid scaffold such as a DNA tetrahedron or origami frame carrying the geometry, since sequences asked to do both tend to do neither reproducibly.

Judged against these principles, no single architecture currently wins on every axis. DNA origami offers the best positional precision and the poorest scalability; random aggregation offers the lowest cost and the poorest reproducibility. The most workable compromise at present is a magnetically separated core-satellite or DNA-tetrahedron assembly carrying an embedded internal standard, which reaches sub-picomolar sen-

sitivity with a signal distribution narrow enough to calibrate, using components that can be produced in batch.

Readiness also differs sharply by application. Nucleic acid detection in buffered or lightly processed samples is closest to deployment, because sequence-defined recognition, amplification chemistry and reporter placement are all mature. Extracellular vesicle and circulating tumor cell assays sit one step behind, held back by capture efficiency and matrix variability rather than by the plasmonics. Live-cell and longitudinal intracellular measurements remain proof-of-concept: probe stability, delivery and the absence of an internal reference make quantitative comparison across cells and across time points the unsolved problem.

Beyond proof-of-concept studies, the focus must now shift toward robust engineering, standardized workflows, clinically relevant benchmarking, and user-friendly interfaces. Achieving this vision will require a multidisciplinary framework in which spectroscopists, nanotechnologists, biomedical engineers, data scientists, clinicians, and regulatory experts work together to translate laboratory advances into practical healthcare solutions. In this sense, the future of aptamer-SERS biosensing will be defined not only by stronger hotspots or lower detection limits, but by the successful alignment of molecular programmability, signal transduction, quantitative robustness, manufacturability, and clinical validation. Overall, programmable DNA aptamer-plasmonic nanocomplexes provide more than a new class of ultrasensitive biosensors: they establish a design framework in which molecular recognition, nanoscale organization, and optical readout can be rationally integrated. By linking programmable architecture to biomedical function and translational readiness, aptamer-SERS technologies are well positioned to contribute not only to next-generation diagnostics, but also to broader advances in precision medicine, functional bioanalysis, and responsive nanomaterials.

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List of Abbreviations

Abbreviation	Explanation	Abbreviation	Explanation
SERS	Surface-enhanced Raman scattering	AI	Artificial intelligence
LSPR	Localized surface plasmon resonance	SELEX	Systematic Evolution of Ligands by Exponential enrichment

TEM	Transmission electron microscopy	RCA	Rolling circle amplification
DONA	DNA origami nanofork antenna	ECL	electrochemi luminescence
CHA	Catalyzed hairpin assembly	PSA	Prostate-specific antigen
SPE	Screen-printed electrodes	PTH	Parathyroid hormone
WGA	Wheat germ agglutinin	ICTR-WHO	International Clinical Trials Registry Platform of World Health Organization
ML	Machine learning	TRL	Technology Readiness Level
GMP	Good Manufacturing Practice	POC	Point-of-care
CNN	Convolutional neural network	SVM	Support vector machine
KNN	k-nearest neighbors	CTCs	Circulating Tumor Cells
MUC1	Mucin-1	bHCR	Branched hybridization chain reaction
TDNs	Tetrahedral DNA nanostructures	LDR	Ligase detection reaction
SNP	Single nucleotide polymorphism	EpCAM	Epithelial cell adhesion molecule
BMSCs	Bone marrow-derived stem cells	UTI	Urinary tract infection
TEXs	Tumor-derived exosomes		

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