Nanoscale Horizons

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal

Cite this: DOI: 10.1039/d5nh00406c

Passive and active biosensing with nucleic acid–protein hybrid nanostructures

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Received 10th June 2025,
Accepted 20th October 2025

DOI: 10.1039/d5nh00406c

rsc.li/nanoscale-horizons

Advanced profiling of multiple biomarkers can individualize patient characterization and empower precision medicine. Conventional diagnostic methods, however, often require extensive processing and lack assay versatility and/or multiplexing capacity to accommodate different biomarkers. To address these challenges, nucleic acid–protein hybrid nanostructures have emerged as a promising technology. These hybrids offer multifaceted versatility. On the component level, they benefit from the inherent structural programmability of nucleic acids and the functional versatility of proteins to accommodate diverse biomarkers; as integrated assemblies, they can operate as passive labeling constructs or active enzymatic machines to meet varying diagnostic needs. In this review, we highlight recent synergistic advances in the molecular configuration and mechanism design of these hybrid systems to measure a broad spectrum of biomarkers, ranging from classical nucleic acid and protein biomarkers to novel modifications and interactions. Finally, we provide an outlook on emerging trends in biomarker discovery and technology development that position nucleic acid–protein hybrids as powerful tools for precision diagnostics.

1. Introduction

Precision medicine holds the potential to revolutionize healthcare by enabling early disease detection, refined classification, and personalized treatment strategies based on an individual's unique biomarker profile.[1] To realize this potential, advanced profiling of multiple biomarkers is needed to fully capture personalized differences across patients.[2] Indeed, myriad disease biomarkers exist—including nucleic acids, proteins, small molecules as well as their novel modifications and interactions—to provide a rich source of molecular information for highly individualized characterization and patient stratification.[3] Nevertheless, conventional diagnostic technologies often fall short in the multiplexed measurement of these classical and novel biomarkers, especially against a vast and complex biological background of clinical samples. Existing technologies commonly have limited assay versatility and/or multiplexing capacity to accommodate diverse biomarkers. For example, nucleic acid-based techniques such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) offer exceptional throughput for multiplexed quantification of target sequences but have limited assay versatility to measure other nucleic acid-based biomarkers (*e.g.*, nucleic acid modifications).[4,5] On the other hand, protein analytical methods including enzyme-linked immunosorbent assays (ELISA) and Western blotting can be designed to address a broader spectrum of target biomarkers (*e.g.*, proteins and protein modifications), but they face limited throughput and cannot be readily multiplexed.[6,7] Importantly, these nucleic acid- and protein-based techniques are inherently incompatible and cannot be readily integrated into a unified platform to exploit respective performance advantages. These challenges underscore the demand for novel diagnostic technologies that can achieve versatile and multiplexed detection of biomarkers in complex clinical samples—an essential step toward realizing precision medicine.[8]

To overcome these challenges, recent advances in biomolecular nanotechnology have focused on leveraging nucleic acids and proteins as complementary functional components to develop new diagnostic platforms.[9] Nucleic acids, including DNA and RNA, offer inherent structural programmability, predictable base-pairing interactions, and extensive chemical modifiability, making them ideal scaffolds to establish reconfigurable and dynamic nanostructures.[10] Drawing on recent

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advances in analytical technologies, nucleic acids can also be analyzed with unprecedented resolution and throughput.[11] On the other hand, proteins provide diverse functionalities, including specific molecular recognition, catalytic activity, and signal amplification, thus enabling specific and multiplexed detection.[12] By combining these two molecular classes as building blocks, nucleic acid–protein hybrid nanostructures harness the structural precision of nucleic acid frameworks alongside the biochemical versatility of protein domains.[13] Various nucleic acid–protein hybrids have been developed. These hybrids are functional assemblies that incorporate nucleic acid components (*e.g.*, DNA/RNA scaffolds, aptamers, or primers) with protein domains (*e.g.*, enzymes, antibodies, or binding proteins) through covalent and/or non-covalent interactions. In so doing, the hybrids integrate recognition elements, structural frameworks, and catalytic modules into unified architectures that can detect a broad spectrum of analytes—from classical nucleic acid and protein biomarkers to novel modifications and interactions—within a single, modular platform. Notably, depending on the assay configuration, these systems can be designed as passive classifiers, leveraging intrinsic recognition and labeling capabilities to identify biomarkers, or as active machines, whose responsive activity can be switched on and off to boost performance and functionality.

While previous reviews have focused primarily on the preparation,[9] and structural diversity[14,15] of nucleic acid–protein hybrid nanostructures, this review provides a distinct perspective on the synergy between hybrid molecular configuration (*i.e.*, components and interactions) and assay design (*i.e.*, operational mechanisms) to achieve various clinical applications. Indeed, the dual-mode operational flexibility—passive and active systems—endows nucleic acid–protein hybrid nanostructures with exceptional versatility and analytical performance to accommodate a broad spectrum of biomarkers with varying properties, thereby enabling direct and informative biomarker detection in complex biological samples for next-generation precision diagnostics. In this review, we discuss how different hybrid configurations enable functional assay mechanisms. Through the development of various passive labeling systems as well as active enzymatic platforms, we explore the expanding utility of nucleic acid–protein hybrids in detecting different biomarkers, including nucleic acids, proteins, small molecules as well as novel modifications and interactions in these biomarkers. Finally, we provide perspectives on emerging opportunities with respect to biomarker discovery, hybrid design and system integration, and discuss how these interdisciplinary developments can empower pre-clinical and clinical applications of hybrid nanostructures for next-generation molecular diagnostics.

2. Nucleic acid–protein hybrid systems

2.1. Molecular components: nucleic acids and proteins

Nucleic acids and proteins are fundamental building blocks of life. While nucleic acids store, encode, and transmit genetic information, proteins carry out diverse structural, catalytic, and regulatory functions.[16,17] Inspired by these natural roles, nanobiotechnology harnessed their unique properties to develop advanced functional systems. For example, by leveraging predictable base-pairing interactions and strand displacement reactions, myriad nucleic acid architectures, including linear DNA/RNA strands, folded aptamers, tetrahedral frameworks, and DNA origami, have been developed with high spatial precision and molecular programmability (Fig. 1(a)).[18–20] On the other hand, native and engineered proteins have been used to achieve diverse functionalities, such as specific molecular recognition, catalytic activity, and signal transduction. These proteins include antibodies, enzymes, binding proteins, and various fluorescent or luminescent reporters.[21,22] Motivated by their complementary properties, nucleic acid–protein hybrids synergize the structural programmability of nucleic acid frameworks with the biochemical versatility of protein domains to develop highly adaptable systems that can recognize a wide range of biomarkers.[23]

2.2. Operational modes: passive *vs.* active systems

Beyond these inherent advantages brought by biological building blocks, nucleic acid–protein hybrids—as thoughtfully configured assemblies—can offer more. Operationally, these hybrid systems can be designed to function through passive or active mechanisms (Fig. 1(b)). Passive systems leverage direct molecular recognition and labeling. For example, antibodies conjugated to DNA scaffolds can report target biomarker abundance, through high-throughput measurement of different target-bound DNA probes, thereby enabling highly multiplexed biomarker detection (Fig. 1(b), top).[24–26] In contrast, active systems leverage dynamic or enzymatic processes to activate signaling. Upon target recognition, an engineered enzyme or conformational switch is triggered, initiating a catalytic cascade that converts an inactive "off" state into a robust "on" signal; this cascading activity can significantly boost detection sensitivity to measure even trace biomarkers against a complex biological background (Fig. 1(b), bottom).[27,28]

2.3. Synergistic designs and expanded capabilities

To achieve these distinct modes of action, different synergistic designs (*e.g.*, component selection and assembly strategies) have been applied to configure functional nucleic acid–protein hybrids (Fig. 1(c) and (d)). Specifically, passive systems are typically constructed through covalent conjugation; these systems employ a variety of techniques, including chemical conjugation (*e.g.*, maleimide–thiol), bio-orthogonal chemistry (*e.g.*, click chemistry), genetic code expansion (GCE) and fusion protein engineering, to achieve stable and robust constructs (Fig. 1(c)).[29–33] Active hybrids, on the other hand, rely primarily on non-covalent interactions to establish switchable assemblies; various reversible interactions including specific ones (*e.g.*, aptamer–protein binding and Ni-NTA/His-tag selectivity) as well as general physicochemical forces (*e.g.*, electrostatic interactions, hydrogen bonding, and hydrophobic effects) have been used to develop stimuli-responsive systems that can modulate both molecular conformation and enzyme activity (Fig. 1(d)).[34–37]

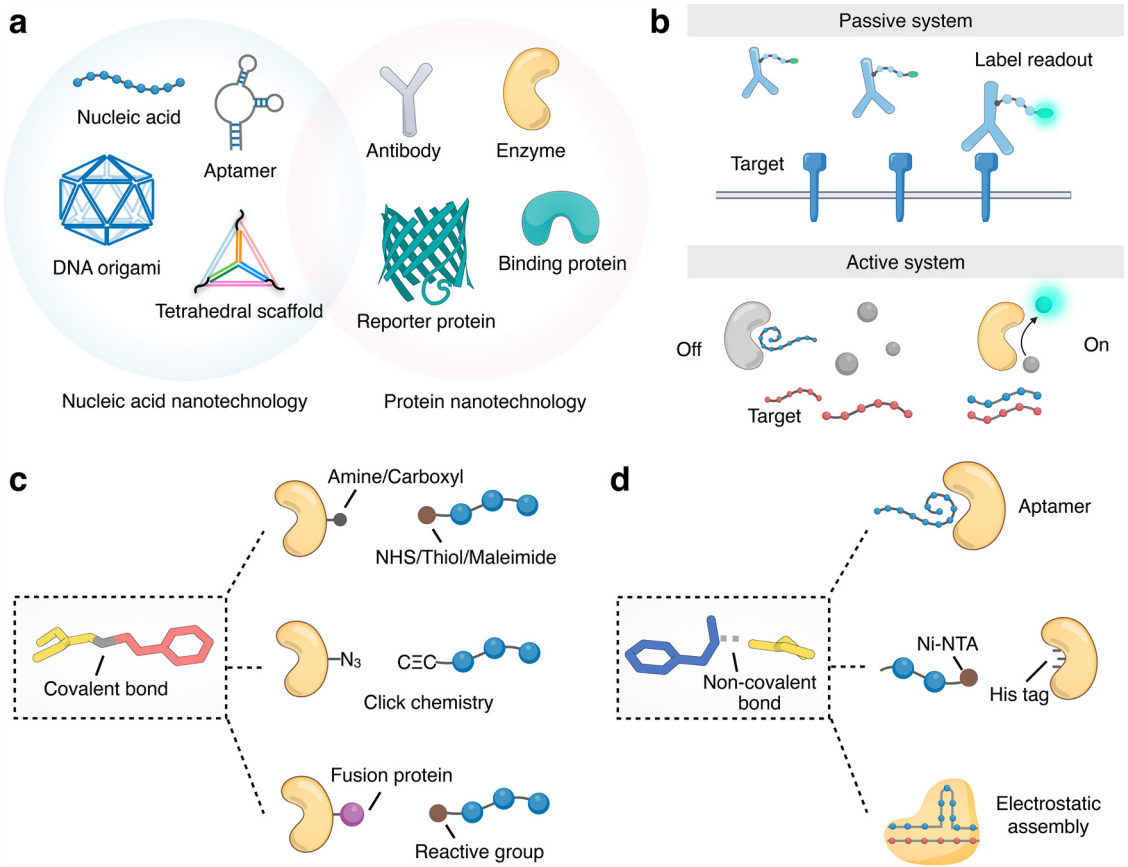

Fig. 1 Molecular configuration and mechanism design of nucleic acid–protein hybrids. (a) Component versatility of nucleic acid–protein hybrids. Benefiting from the inherent diversity of their biological building blocks—the structural programmability of nucleic acids and the functional versatility of proteins—various nucleic acid–protein hybrids can be developed. (b) These nucleic acid–protein hybrid systems function in passive or active modes to measure a broad spectrum of biomarkers. Passive systems detect biomarker targets through direct molecular recognition and labeling. For instance, antibodies conjugated to nucleic acid scaffolds can directly target and report biomarkers (top). In contrast, active systems utilize dynamic or enzymatic processes to achieve switchable behaviors, transitioning from an "off" state to an "on" state in response to target-induced activation or molecular reconfiguration (bottom). (c) and (d) These functional modes are enabled by distinct assembly strategies: (c) passive systems commonly employ covalent conjugation to form stable and irreversible linkages between nucleic acids and proteins. Typical methods include chemical conjugation (*e.g.*, maleimide–thiol, NHS–ester, and amine–carboxylate reactions), bio-orthogonal chemistry (*e.g.*, click chemistry), genetic code expansion (GCE), and fusion protein engineering. (d) Active systems rely primarily on non-covalent interactions—specific interactions (*e.g.*, aptamer–protein binding and His-tag/Ni-NTA affinity) or general physicochemical forces (*e.g.*, electrostatic interactions, hydrogen bonding, and hydrophobic effects)—to achieve reversible and switchable assemblies.

Collectively, nucleic acid–protein hybrid nanostructures provide a versatile and powerful framework for diagnostic development. These hybrids not only benefit from the inherent properties of their biological building blocks (*i.e.*, programmability of nucleic acids and functional versatility of proteins) but also provide mechanistic versatility as functional assemblies. This diversity provides exceptional opportunities for clinical applications, especially against a vast and complex biological background of patient samples. For example, the use of orthogonal DNA probes in passive labeling systems improves their compatibility with native patient samples, by generating amplified signals with minimal interferences from abundant biological background DNA.[38] Likewise, active systems with carefully designed transduction mechanisms can be applied directly to native patient samples; these are turned on when bound to target biomarkers but remain inactive against the complex background of neat patient samples.[39] Importantly, the programmable versatility of nucleic acid–protein hybrid systems enables not only multiplexed measurements of a broad spectrum of biomarkers but also enhanced clinical diagnostic accuracy with minimally processed clinical samples.[15] In the following sections, we describe recent advances in the molecular configuration and mechanism design of these hybrid systems that enable high-performance detection of a wide range of biomarkers—from classical nucleic acid and protein biomarkers to novel targets such as modifications and interactions.

3. Detection of nucleic acids and modifications

Nucleic acid-based biomarkers, including DNA and RNA sequences as well as epigenetic modifications, play crucial roles in regulating gene expression, cellular function, and disease progression.[40,41]

Although conventional technologies like PCR and NGS can enable sensitive detection of nucleic acid sequences, they require complex sample processing and have limited assay versatility to measure other nucleic acid-based biomarkers (*e.g.*, epigenetic modifications). Leveraging their target programmability and functional capability, nucleic acid–protein hybrids offer a powerful strategy for detecting nucleic acid biomarkers in a multifaceted manner.[42]

3.1. Hybrid systems for nucleic acid detection

3.1.1. DNA–enzyme hybrid systems. To enable the direct detection of nucleic acid sequences, Ho *et al.* recently developed a DNA–enzyme hybrid nanostructure named enzyme-assisted nanocomplexes for visual identification of nucleic acids (enVision) that seamlessly combines target recognition and signal amplification within a unified construct (Fig. 2(a)).[43] In this active system, a DNA aptamer binds specifically to Taq DNA polymerase, inhibiting its enzymatic activity in the absence of the target nucleic acid. Upon target recognition, the aptamer hybridizes with the target nucleic acid, releasing the polymerase and restoring its activity. The reactivated polymerase then incorporates biotin-labeled nucleotides, recruiting horseradish peroxidase (HRP) for a colorimetric readout. The technology is notable for its modular integration of target recognition with a unified signal amplification; such design enables rapid prototyping of new assays and multiplexed detection, as new aptamer–polymerase switches can be easily developed and detected through the universal signaling mechanism. For switch design, as the system relies on aptamer–polymerase inhibition, it is anticipated that the relative component ratio in the hybrid assembly could significantly influence the overall activation performance. Indeed, building upon the initial design, the team further enhanced the technology sensitivity by fine-tuning the DNA-to-enzyme ratio to establish a metastable hybrid mixture that could be easily activated by even trace target amount.[44,45] Extending this concept, by employing G-quadruplex DNAzymes immobilized on a screen-printed electrode for signal amplification, the system was further synergistically coupled to establish a responsive equilibrium through the dual-enzyme network. In this work, target RNA binding triggers a conformational transition that initiates a multi-step catalytic cascade, amplifying electrochemical signals to achieve single-copy sensitivity in clinical sample lysates for direct SARS-CoV 2 detection (Fig. 2(b)).[46] The study illustrates an interesting example on the synergistic integration of multiple DNA–enzyme hybrids; as each hybrid possesses its unique responsive equilibrium, the work highlights not only the complex dynamics but also engineering opportunities to achieve exceptional performance based on multiple hybrids.

3.1.2. CRISPR-based hybrid systems. As another example of nucleic acid–protein hybrid systems, CRISPR-based platforms, which combine guide RNA (gRNA) components with Cas nuclease proteins into non-covalent nanostructures, can couple sequence recognition with catalytic cleavage activity for ultrasensitive RNA detection.[47,48] Pardee *et al.* demonstrated an active Cas9–gRNA hybrid system in which the enzyme selectively cleaves a protospacer adjacent motif (PAM) sequence associated with Zika virus RNA, integrated with a synthetic toehold switch designed to initiate nucleic acid sequence-based amplification (NASBA) (Fig. 2(c)).[49] When the target PAM sequence is present, Cas9 cleaves the RNA, producing a truncated RNA that fails to activate the NASBA reaction; without the PAM, the toehold switch remains intact, enabling downstream amplification. This platform is notable for its adaptability to recognize diverse nucleic acid targets, offering precise sequence recognition and highly specific enzymatic activity. With its involvement of multiple reactions (*e.g.*, NASBA and Cas9 activity), complex interplay must be carefully controlled and optimized. To ease such dynamics, in a separate study, the Cas9–gRNA complex was immobilized on a graphene field-effect transistor (FET), allowing label-free electrical detection without pre-amplification.[50]

In addition to Cas9, other Cas enzymes, such as Cas12a, Cas13a, and Csm6, exhibit collateral cleavage activity: upon specific target recognition, these enzymes become catalytically activated to indiscriminately cleave nearby single-stranded reporter sequences (Fig. 2(d)).[51] This mechanism underlies the platform named specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) developed by Gootenberg *et al.* Following isothermal amplification, target binding to Cas12 or Cas13 activates trans-cleavage of fluorescent or colorimetric reporters, enabling highly sensitive (attomolar) and multiplexed detection with single-nucleotide resolution. More recently, Patchsung *et al.* adapted the SHERLOCK technology for point-of-care use with lateral flow assays,[52] and other efforts have further advanced the technology to enable amplification-free RNA detection and quantification.[53] These studies illustrate key advantages of the collateral cleavage by Cas systems, including single amplification and multiplexed detection. Further developments through platform integration (*e.g.*, lateral flow assays and microfluidics) could enhance assay robustness and accessibility for various clinical applications.

3.2. Hybrid systems for detecting RNA–protein interactions and epigenetic modifications

Beyond quantification of these classical nucleic acid biomarkers, different hybrid systems have been employed to investigate RNA–protein interactions and epigenetic modifications. In one approach based on Förster resonance energy transfer (FRET), the Spinach RNA aptamer is conjugated to a fluorescently labeled PP7 coat protein (Fig. 2(e)).[54] Upon target RNA binding, the two fluorophores are brought into proximity, resulting in Spinach fluorescence quenching and enabling real-time, label-free monitoring of RNA–protein binding kinetics in live cells. This passive detection strategy streamlines assay design while facilitating dynamic studies of RNA regulatory processes *in situ*. On the other hand, to detect epigenetic modifications, antibodies against N^6-methyladenosine (m^6A) and N^1-methyladenosine (m^1A) have been immobilized onto DNA scaffolds *via* microneedle arrays (Fig. 2(f)).[55] When two antibodies simultaneously recognize adjacent methylation sites on the same RNA sequence, the DNA scaffold undergoes

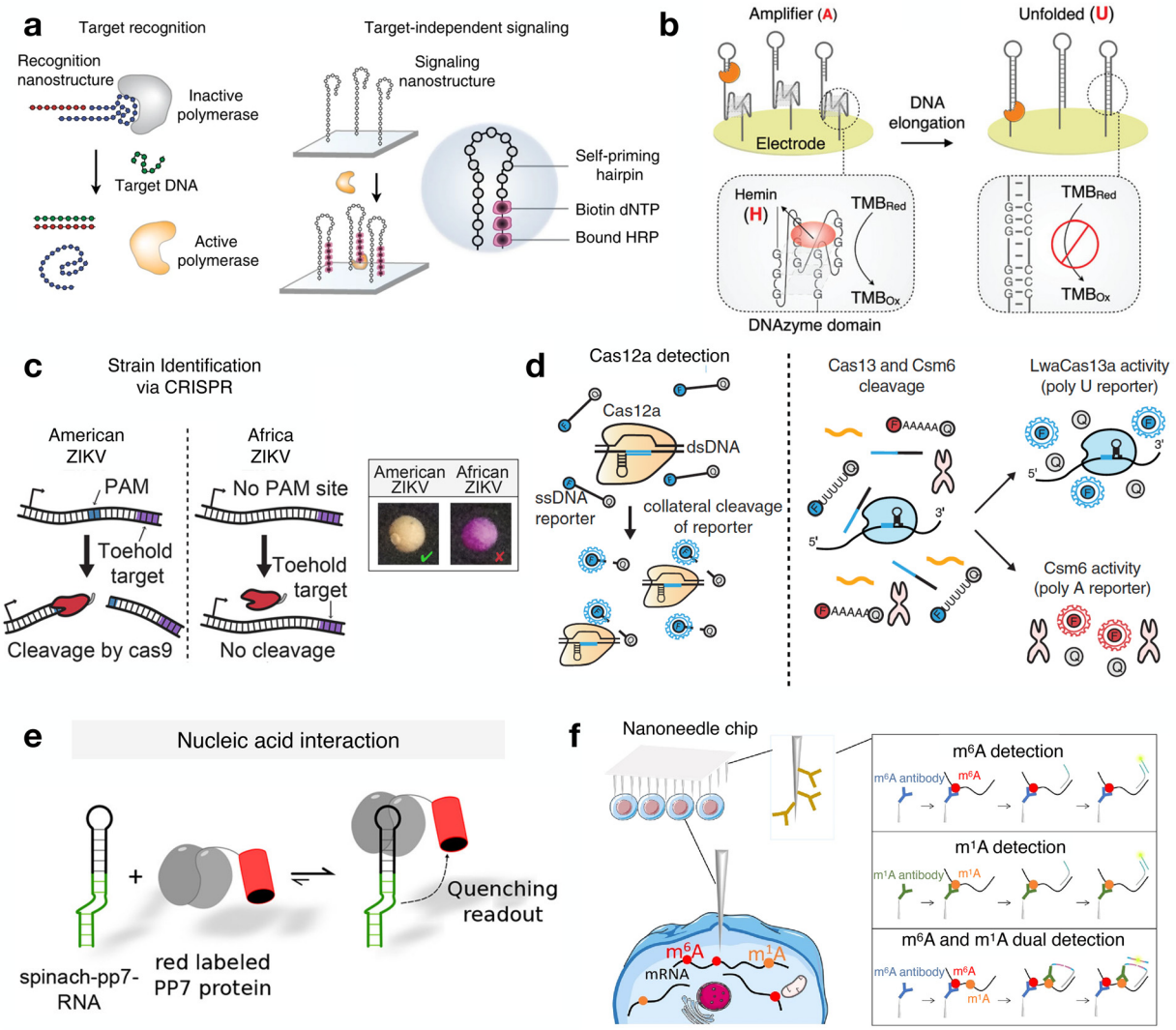

Fig. 2 Detection of nucleic acids and modifications. (a) Enzyme-assisted nanocomplexes for visual identification of nucleic acids (enVision). This assay integrates target recognition and signal amplification within a unified construct. An aptamer initially binds and inhibits DNA Taq polymerase, forming the recognition element and preventing enzyme activity in the absence of target. Upon target recognition, the aptamer hybridizes with the target sequence, releasing the polymerase to elongate a self-priming signaling nanostructure and incorporate biotin-labeled dNTPs. These labeled nucleotides subsequently conjugate with horseradish peroxidase (HRP) to form the signaling component, enabling colorimetric detection on an immobilized nanostructure. Reproduced from ref. 43 with permission from Springer Nature, copyright 2025. (b) Architecture of the synergistic coupling of responsive equilibrium in enzymatic network (SCREEN). The amplification network is constructed from an amplifier DNA nanostructure immobilized on a screen-printed electrode, comprising a polymerase-binding hairpin domain and a G-quadruplex-assembled DNAzyme domain complexed with hemin. Before elongation, the DNAzyme catalyzes hemin-mediated TMB oxidation. Upon target recognition, polymerase-mediated elongation unfolds the G-quadruplex, disrupting DNAzyme activity and switching off the colorimetric signal. Reproduced from ref. 46 with permission from John Wiley and Sons, copyright 2025. (c) CRISPR-Cas9-based strain identification of Zika virus (ZIKV). This active system consists of Cas9 protein and a synthetic toehold switch. The Cas9 protein selectively cleaves a protospacer adjacent motif (PAM) sequence present in the American ZIKV strain, thereby inhibiting activation of the integrated synthetic toehold switch and preventing nucleic acid sequence-based amplification (NASBA). In contrast, the African ZIKV strain lacks the PAM site, avoiding cleavage and allowing NASBA-triggered colorimetric signal generation on a paper-based platform with single-nucleotide resolution. Reproduced from ref. 49 with permission from Elsevier, copyright 2025. (d) CRISPR-Cas12 and Cas13 systems for nucleic acid sensing. These active systems leverage the collateral cleavage activity of Cas12, Cas13a, and Csm6 for multiplexed detection. Cas12a binds to double-stranded target DNA and activates collateral cleavage of a fluorogenic single-stranded DNA reporter (left). Whereas Cas13 and Csm6 cleave poly-U and poly-A RNA reporters, respectively, upon specific RNA target recognition, enabling orthogonal and multiplexed signal amplification (right). Reproduced from ref. 51 with permission from The American Association for the Advancement of Science, copyright 2025. (e) Hybrid FRET-based platform for probing nucleic acid–protein interactions. A Spinach RNA aptamer is conjugated to a pp7 RNA motif, forming a Spinach-pp7 RNA complex. Upon target RNA binding, red-labeled PP7 protein is brought into proximity with the aptamer, leading to Spinach fluorescence quenching and enabling real-time monitoring of RNA–protein interaction dynamics. Reproduced from ref. 54 with permission from American Chemical Society, copyright 2025. (f) Hybrid nanoneedle chip for simultaneous detection of RNA epigenetic modifications. Antibodies against N^6-methyladenosine (m^6A) and N^1-methyladenosine (m^1A) are immobilized onto DNA scaffolds *via* microneedle arrays, enabling selective and simultaneous recognition of m_6A and m^1A modifications on mRNA. Subsequent proximity ligation of attached nucleic acid handles enables amplification and discrimination of m_6A-only, m^1A-only, and dual-modified transcripts, allowing comprehensive epitranscriptomic profiling at the single-cell level. Reproduced from ref. 55 with permission from American Chemical Society, copyright 2025.

ligation, producing a multiplexed, spatially resolved readout of RNA methylation patterns. This passive system synergistically combines the molecular recognition capabilities of antibodies with the programmability and spatial precision of nucleic acid nanostructures, yielding a versatile platform for comprehensive epitranscriptomic analysis. Collectively, these active and passive nucleic acid–protein hybrid platforms showcase the synergistic integration of biomolecular components to enable advanced detection of nucleic acid features across diverse diagnostic contexts. These features, including RNA–protein interactions and epigenetic modifications, are not only innovative biomarkers but also challenging ones that cannot be readily measured by conventional methods. Further hybrid developments could thus accelerate the scientific and clinical reach of such biomarkers, by facilitating accessible measurements in different clinical samples and achieving informative analysis at an unprecedented resolution and scale.

4. Detection of proteins and interactions

Proteins and their interactions reveal cellular states and regulatory pathways, making them critical biomarkers for disease diagnosis and mechanism elucidation. However, conventional protein detection methods, such as ELISA and Western blotting, have limited analytical performance, especially for low-abundance biomarkers,[56,57] and cannot be effectively multiplexed,[58] thereby limiting their throughput and restricting comprehensive analysis.

4.1. Hybrid systems for detection of protein abundance

4.1.1. Immuno-PCR-based hybrid systems. To address some of these challenges, nucleic acid-mediated signal amplification strategies have been incorporated to achieve immunoassays with enhanced sensitivity, especially for detecting low-abundance protein targets.[59] Early approaches, such as immuno-polymerase chain reaction (immuno-PCR) and immuno-rolling circle amplification (immuno-RCA), rely on conjugating detection antibodies to DNA tags that can be exponentially amplified through PCR[56] or RCA.[57] Specifically, conventional immuno-RCA employs copper-free click chemistry to conjugate detection antibodies to DNA tags at a 1 : 1 ratio, ensuring each target binds to a single DNA tag. While such methods offer good sensitivity due to their ability for exponential signal amplification, they can suffer from limited antibody conjugation efficiency. To address the challenge, Yan *et al.* immobilized the detection antibody on gold nanoparticles, whose large surface area accommodates more DNA tags, thereby improving the conjugation efficiency and achieving further signal amplification and attomolar sensitivity.[60] To further enhance the signal-to-noise ratio, especially in samples with strong auto-fluorescence background, Jiang *et al.* designed a method involving optical tweezer-assisted immuno-RCA on beads, in which sandwich complexes formed by capture antibody, target protein, and DNA primer–conjugated detection antibody trigger RCA, producing amplified fluorescence that is stably detected through trapping of the individual beads by optical tweezers (Fig. 3(a)).[61] This optical tweezer approach provides additional selectivity and reduces variability arising from heterogeneous reaction environments. Nevertheless, its requirement for specialized optical instrumentation may limit the technology's accessibility, especially for resource-limited clinical applications.

4.1.2. Tyramide signal amplification-based hybrid systems. Beyond antibody-based capture, researchers have leveraged programmable, high-specificity aptamers and combined them with the catalytic activity of enzymes to achieve highly adaptable and sensitive protein detection. For instance, Huang *et al.* combined an anti-SARS-CoV-2 nucleocapsid protein aptamer with HRP to enable activatable tyramide signal amplification (TSA) on paramagnetic beads (Fig. 3(b)).[62] When the aptamer–HRP conjugate binds to the captured target antigen, HRP catalyzes the H_2O_2-mediated activation of tyramide derivatives. These activated tyramide derivatives covalently bind to tyrosine residues on the target protein and generate a fluorescent signal with minimal background, enabling flow cytometry analysis. This system couples the specificity of aptamers with enzymatic amplification, offering modularity and low background in fluorescence detection. By simply altering the aptamer sequence, aptamer–enzyme systems can also be applied for multiplexed proteomic profiling in biological fluids. Li *et al.* developed a platform that harnesses the high specificity of aptamers to "fish" multiple serum biomarkers *via* a nanoparticle–protein corona, and then transduces each aptamer–protein binding event into a CRISPR/Cas12a-mediated trans-cleavage signal, enabling orthogonal, multiplexed fluorescence readout with high sensitivity and throughput (Fig. 3(c)).[63] The modularity and signal amplification proffered by CRISPR–Cas make this system highly promising for multiplexed profiling. Further implementation and optimization of the Cas activity across different channels will enable not only multiplexed detection but also quantitative accuracy.

4.2. Hybrid systems for detection of protein–protein interactions and protein modifications

In addition to measuring protein abundance, nucleic acid–protein hybrid nanostructures have also been employed to identify protein–protein interactions and detect protein modifications. For example, Gullberg *et al.* developed a proximity ligation assay (PLA) platform in which antibodies conjugated to unique DNA oligonucleotides are brought into proximity upon binding to interacting proteins.[64] This co-localization enables DNA ligation, generating an amplifiable DNA reporter that reflects binary protein interactions, although reliance on dual antibody proximity may limit applicability to certain sterically hindered complexes. Beyond binary protein interactions, to assess multi-component protein assemblies, Liu *et al.* introduced mid-strand DNA barcodes on antibodies *via* polyethylene glycol (PEG) linkers (Fig. 3(d)).[65] Each antibody is tagged with a unique identifier sequence along with a universal overlap region. When multiple antibodies bind to components of a protein complex, added complementary "barcode growth"

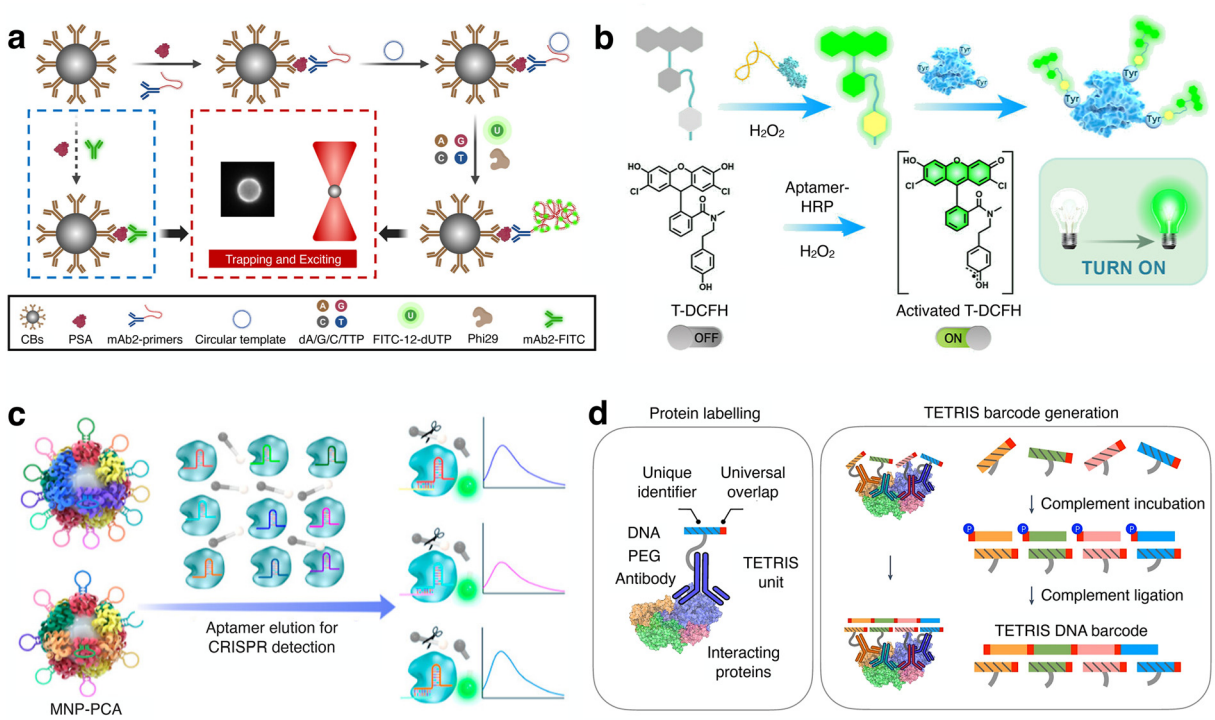

Fig. 3 Detection of proteins and interactions. (a) Optical tweezer-assisted immuno-RCA on beads. In this design, sandwich complexes are formed with a capture antibody (mAb1), a DNA primer-conjugated detection antibody (mAb2), and the target protein. Upon target recognition, the sandwich complex assembles on the bead, wherein rolling circle amplification (RCA) is initiated in the presence of a circular template, phi29 DNA polymerase, dNTPs, and FITC-labeled nucleotides (FITC-12-dUTP) to achieve fluorescence signal amplification for highly sensitive protein detection. The trapping and excitation of individual beads by optical tweezers enable stable signal output. Reproduced from ref. 61 with permission from American Chemical Society, copyright 2025. (b) Aptamer-based activatable tyramide signal amplification (TSA) for low-background detection of SARS-CoV-2 nucleocapsid protein. This active system combines an anti-SARS-CoV-2 nucleocapsid protein aptamer with HRP to enable activatable TSA on paramagnetic beads. Upon target protein binding with the aptamer–HRP conjugate, HRP catalyzes the H_2O_2-mediated activation of a tyramide derivative (T-DCFH). The activated T-DCFH molecules then covalently bind to tyrosine residues on the target protein and generate fluorescence signals. Reproduced from ref. 62 with permission from American Chemical Society, copyright 2025. (c) CRISPR/Cas12a-based orthogonal multiplexed aptamer sensing for proteome profiling. This platform harnesses high-specificity aptamers to bind multiple serum biomarkers through a nanoparticle–protein corona (MNP–PC), and then transduces each aptamer–protein binding event into CRISPR/Cas12a-mediated trans-cleavage signal, enabling orthogonal, multiplexed fluorescence readout with high sensitivity and throughput. Reproduced from ref. 63 with permission from American Chemical Society, copyright 2025. (d) Direct mapping of protein interactions of different orders using DNA barcodes. Unique identifier sequences are attached to antibodies *via* polyethylene glycol (PEG) linkers. When multiple antibodies bind to components of a protein complex, added complementary DNA strands hybridize bidirectionally and form a DNA barcode through ligation of fragments. This DNA barcodes is then released and sequenced to decode the composition and stoichiometry of binary, ternary, or higher-order interactions based on combinatorial patterns. Reproduced from ref. 65 with permission from Springer Nature, copyright 2025.

strands hybridize bidirectionally, and DNA ligase seals adjacent fragments into a contiguous barcode that enables decoding of the composition and stoichiometry of binary, ternary, or higher-order interactions based on combinatorial barcode patterns. This approach allows high-throughput profiling of complex interactions, but it requires robust bioinformatics to decode barcodes and quantify different target complexes. Moreover, by exploiting its proximity-dependent detection capability, PLA-based nucleic acid–protein hybrid systems have also been applied to identify post-translational modifications in proteins. For instance, Chen *et al.* designed a PLA-driven sensing platform to detect protein modified by small ubiquitin-like modifier (SUMO), wherein the SUMO modification is tagged with Glutathione *S*-transferase (GST) protein to facilitate subsequent labeling with a DNA-conjugated anti-GST antibody.[66] Through binding of another DNA-linked antibody to the target protein, a pair of antibodies in proximity will be formed in the presence of protein modification, enabling the generation of amplified signal *via* DNA ligation and PCR. This application exemplifies the versatility of PLA in probing dynamic protein states; further enhancement to its responsiveness could enable more real-time readouts.

5. Detection of small molecules and metabolites

As promising biomarkers, small molecules and metabolites play central roles in cell signaling and metabolic regulation. When measuring these biomarkers, conventional analytical methods such as high-performance liquid chromatography and mass spectrometry often require labor-intensive workflows

including chromatographic separation or chemical derivatization to enrich and analyze the biomarkers from complex matrices; these approaches thus have limited clinical utility and scalability.[67] In contrast, nucleic acid–protein hybrid systems provide a versatile platform to enable direct and multiplexed detection of small molecules.

5.1. Transcription factor-based hybrid systems

Various molecular hybrids have been developed in cells and acellular conditions to measure small molecules. In cells, for example, transcription factor (TF)-based sensors such as the *E. coli* LacI repressor have been implemented; these sensors leverage ligand-induced structural changes—such as dissociation from their DNA operator sequences upon binding to isopropyl-β-D-thiogalactopyranoside (IPTG) or lactose—to activate downstream reporter expression.[68] Similarly, engineered AraC systems and TetR-family transcription factors can translate ligand binding (*e.g.*, arabinose or small-molecule inducers) into transcriptional outputs.[69,70] These whole-cell TF sensors offer high modularity and can incorporate logical control circuits, making them valuable for dynamic metabolic feedback and biosynthetic pathway regulation. However, they can suffer from leaky basal expression, narrow dynamic range, and host-specific effects—especially when transferred from bacteria to eukaryotes. Optimization strategies such as tuning TF levels, ribosome binding sites (RBS) and promoters may help, but are typically labor-intensive and system-specific.[71,72] To overcome the constraints of cellular complexity and regulatory burden, acellular molecular hybrids have also been assembled. For example, a platform termed RNA output sensors activated by ligand induction (ROSALIND) has been demonstrated to leverage cell-free transcription and RNA aptamers for modular and field-deployable detection of water contaminants (Fig. 4(a)).[73] ROSALIND benefits from room-temperature stability and simple modularity—no live cells are required, facilitating decentralized applications. Its use of allosteric transcription factors enables detection across diverse targets (*e.g.*, metals, antibiotics, small organics). It is also anticipated that further developments to protect enzymatic degradation under real-world conditions will enhance not only its analytical performance but also its long-term field stability.[74]

5.2. Riboswitch- and FRET-based hybrid system

Beyond cell-free transcription systems, riboswitch-based systems also offer compact detection strategies through passive RNA–ligand interactions. In one example, an *S*-adenosylmethionine (SAM)-responsive riboswitch has been developed; the switch undergoes metabolite-dependent structural rearrangements, upon binding with SAM, to regulate transcription of fluorescent RNA reporters.[75] Similarly, RNA aptamers, such as those designed for theophylline or ATP, can be engineered to regulate gene expression by controlling accessibility of the ribosome-binding site (Fig. 4(b)).[76] These sensors are well-suited for high-throughput screening due to their small size and minimal requirements. Nonetheless, their low activation remains a key limitation, as modest signal changes are commonly observed unless the systems are implemented with rigorous structural or sequence optimization.[77,78] To overcome these signal limitations and enable more robust outputs, researchers have developed composite hybrids that incorporate biotic and abiotic components (*e.g.*, fluorescent reporters). These composite hybrids can effectively transduce target binding into conformational or proximity changes between fluorophore–quencher pairs to result in measurable fluorescence signals (Fig. 4(c)).[79] As an example, cortisol dynamics have been monitored in unprocessed human blood using a DNA–FRET reporter and an evanescent field-coupled optical fiber, achieving continuous, reagent-free sensing with 100 nM sensitivity and 5-minute resolution. As a result, the technology enables real-time, label-free and continuous detection directly in complex biofluids; nevertheless, its need for specialized optical instrumentation may limit its scalability, especially for point-of-care applications.[80]

5.3. DNA origami-confined hybrid systems

In addition to these passive systems that recognize target-induced conformational changes, active hybrid systems with responsive enzyme cascades have been developed to enable efficient signal transduction and amplification. While multi-step catalytic cascades (*i.e.*, based on tandem activities of enzyme pairs such as glucose oxidase (GO_x)/HRP and alcohol dehydrogenase/lactate dehydrogenase (ADH/LDH)[81]) offer powerful signal amplification for small molecule detection, their efficiency is often limited in traditional assays, primarily due to low substrate channeling across freely diffusing enzymes.[82] To address this challenge, researchers have turned to the programmable assembly of oligonucleotides into two- and three-dimensional DNA nanostructures, such as origami sheets,[83] tetrahedra,[20] and buckyballs,[84] to scaffold and organize enzyme pairs at the nanoscale.[85] In one example, Yan *et al.* built a three-dimensional ''caged'' DNA origami to co-localize GO_x and HRP within a confined 20 nm compartment, achieving more than an 8-fold enhancement in cascade activity compared to diffusive systems (Fig. 4(d)).[86] These DNA scaffolds provide precise spatial control and tunable stoichiometry, thus significantly improving the catalytic efficiency. However, challenges such as structural fragility, batch-to-batch variability due to misfolding, and reliance on high-purity DNA and conjugation reagents can continue to complicate reproducibility and scalability.[87]

6. Discussion

Although conventional analytical technologies (*e.g.*, PCR and ELISA) have been widely employed in both basic research and molecular diagnostics, they require extensive sample processing and suffer from limited assay versatility and/or multiplexing capacity to accommodate diverse biomarkers, especially against a complex biological background of clinical samples.[88] To address these challenges, nucleic acid–protein hybrid systems have emerged as a powerful platform for direct, multiplexed and informative molecular analysis. Leveraging the structural

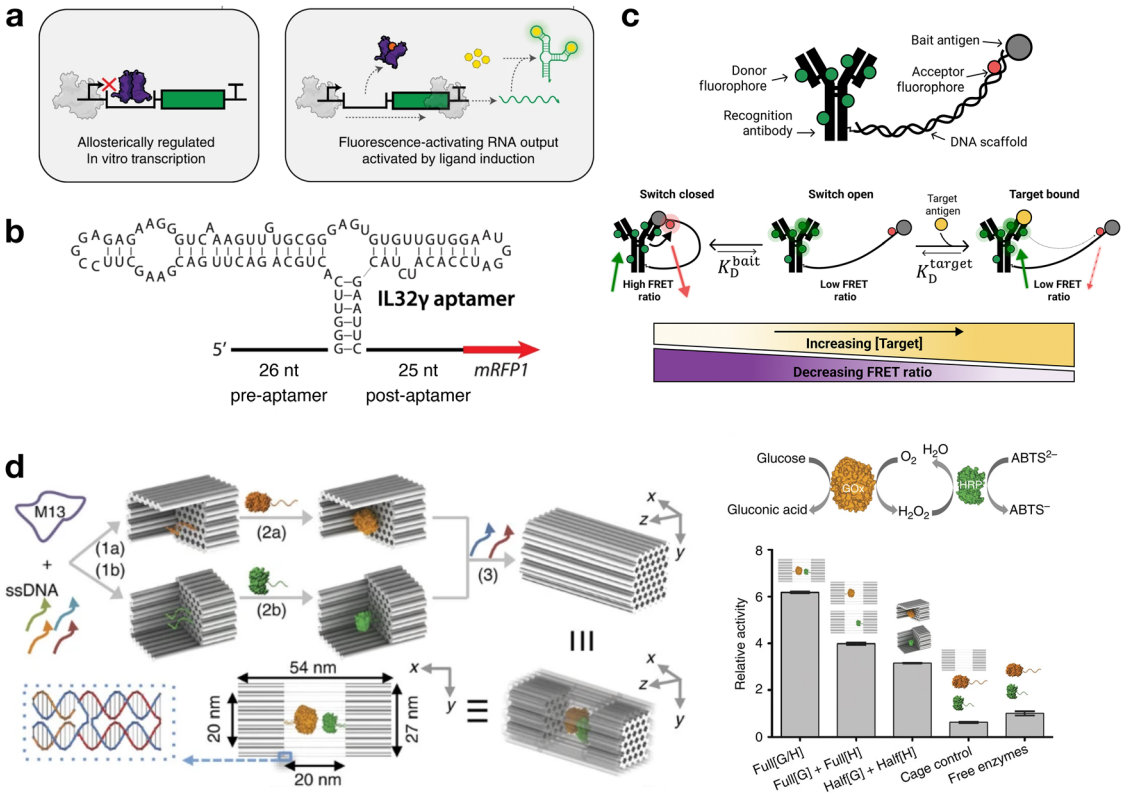

Fig. 4 Detection of small molecules and metabolites. (a) Transcription factor-based hybrid sensor for modular and fieled-deployable detection of water contaminants. This platform comprises three programmable components: a highly processive RNA polymerase, allosteric transcription factors, and synthetic DNA transcription templates. These elements work in concert to allosterically regulate the *in vitro* transcription of a fluorescence-activating RNA aptamer. In the absence of target analyte, transcription is repressed; upon analyte binding, transcription is activated, resulting in a measurable fluorescence signal. Reproduced from ref. 73 with permission from Springer Nature, copyright 2025. (b) Riboswitch-based hybrid sensors. In these systems, RNA folds into a default conformation that permits transcription or translation. When analyte concentration exceeds a threshold, ligand binding induces a structural rearrangement, leading to an alternative RNA conformation that modulates output, such as the expression of a fluorescent reporter. Reproduced from ref. 76 with permission from Springer Nature, copyright 2025. (c) Antibody–DNA FRET switch for real-time molecular sensing. This hybrid system integrates the target recognition capability of an antibody with the programmability of DNA scaffold to form a dynamic FRET-based molecular switch. A fluorophore-labeled antibody is tethered to a complementary bait molecule *via* DNA containing a quencher or FRET acceptor. Upon target binding, conformational changes disrupt the intramolecular antibody–bait interaction, altering fluorophore proximity and shifting the FRET signal. This hybrid design enables real-time, reagent-free quantification of small-molecule targets (*e.g.*, cortisol) in complex biological fluids, showcasing the synergistic strengths of protein recognition and nucleic acid-based structural control. Reproduced from ref. 79 with permission from The American Association for the Advancement of Science, copyright 2025. (d) DNA origami-confined enzyme cascade for glucose detection. This nucleic acid–protein hybrid system spatially organizes a bienzyme cascade, glucose oxidase (GO_x) and horseradish peroxidase (HRP), within a self-assembled 3D DNA origami nanocage (left). Each origami subunit is site-specifically functionalized with one enzyme *via* DNA conjugation, and the subunits are assembled into a confined volume. Compared to controls, including separated cages with individual enzymes, split subunits carrying GO_x or HRP, and free enzyme mixtures with or without vacant origami cages, the confined cascade demonstrates enhanced catalytic output due to spatial proximity and improved substrate channeling (right). Reproduced from ref. 86 with permission from Springer Nature, copyright 2025.

programmability of nucleic acids and the functional diversity of protein components, these hybrid systems can be flexibly designed in passive or active modes to achieve high-performance detection of a wide range of biomarkers—from classical nucleic acid and protein biomarkers to novel modifications and interactions—at an unprecedented resolution and throughput.

6.1. Biomarker discovery and validation

Moving forward, nucleic acid–protein hybrid systems present significant opportunities for biomarker discovery and validation, especially in uncovering novel biomolecular and biophysical biomarkers against complex biological backgrounds. On the biomolecular front, myriad biomarkers with distinct composition and structural diversity have emerged with promising potential to enable earlier detection and more accurate disease stratification. These include cell-surface glycans, whose branching patterns and linkage isomers have been implicated in metastatic progression but remain evasive by existing methodologies, as well as noncoding RNAs, which can adopt complex tertiary folds (*e.g.*, pseudoknots and kissing loops) that govern ligand sensing and translational control but cannot be effectively characterized by linear PCR-based detection methodologies.[89,90] To address these biomarker challenges, nucleic acid–protein hybrid systems with their structural and functional versatility can offer attractive approaches, for

example, through the development of DNA–lectin chimeras that can recognize different glycan forms and structure-selective RNA aptamer fused to an activatable DNA hairpin switch.[91,92] On the biophysical front, new biomarkers including the nanoscale size distribution of extracellular vesicles[93] and piconewton-scale forces exerted at focal adhesions[94] are being extensively explored; while these biomarkers commonly require sophisticated measurement instrumentation (*e.g.*, vesicle flow cytometer and traction force microscopy), programmable nucleic acid–protein hybrid systems with superior size-tunability and mechanoresponsiveness are poised to provide refreshing solutions that not only bypass extensive instrumentation but also empower clinical translation of these biomarkers.[95]

6.2. Technology development and translation

To realize these opportunities, technological advances will continue to drive the evolution of nucleic acid–protein hybrid systems through innovative material development and system-level integration. From the perspective of nucleic acid, the incorporation of innovative and reproducible nucleic acids will not only expand the functional diversity but also robust performance of hybrids. For example, while nucleic acid-based aptamers often offer high-affinity and tunable components for assembling different hybrids, they can be sensitive to various environmental conditions (*e.g.*, buffer composition, ionic strength and temperature), suffer from batch-to-batch variability and require additional reproducibility tests to validate their conformation and performance.[96,97] To address the challenges, the incorporation of chemically modified nucleotides and synthetic backbones (*e.g.*, L-DNA, peptide nucleic acid (PNA), and xenonucleic acids) has shown to enhance the stability, biocompatibility, and functional versatility of DNA devices in complex environment.[98–100] Beyond static structures, dynamic DNA configurations such as walkers, tweezers, and strand-displacement networks allow responsive and programmable behaviors, such as time-gated sensing, sequential signal processing, and conditional actuation.[101–103] Concurrently, advances in *de novo* protein design, fusion-protein engineering, genetic code expansion (*e.g.*, nanobodies,[104] DARPins,[105] and noncanonical amino acids[106]), and directed enzyme evolution have significantly expanded the repertoire of detectable targets. These innovations have not only introduced conformational switches and catalytic amplification modules to tackle challenges in specificity, turnover, and signal transduction, but also enabled the design of entirely new-to-nature proteins capable of detecting rare and previously inaccessible biomarkers. At the system level, nucleic acid–protein hybrid systems could adopt cascading architectures that leverage DNA's programmability and spatial precision alongside engineered protein functionalities, enabling modular and adaptable platforms. Molecular cascades—employing mechanisms such as strand displacement, enzymatic turnover, or hybrid logic—are poised to tightly integrate recognition, signal transduction, and amplification into cohesive, streamlined processing units.[107] Finally, molecular–hardware cascading will link these biochemical outputs directly with physical devices (*e.g.*, electrochemical sensors that measure electrical signals and heat transfer sensors that measure changes in thermal resistance) to enable direct transduction and real-time readout.[3,108] Integration with microfluidic technologies could further boost throughput and scalability.[109] For instance, droplet-based microfluidic platforms could encapsulate patient samples with barcoded DNA–lectin origami sensors,[110] enabling high-throughput, multiplexed glycan profiling. Together, these advances promise to transform nucleic acid–protein hybrids into programmable, device-integrated tools for next-generation biomarker discovery and precision diagnostics.

Conflicts of interest

There are no conflicts to declare.

Data availability

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

Acknowledgements

Z. Yu, Y. Chen and H. M. Tan contributed equally to this work. This work was supported in part by funding from the National University of Singapore (NUS), the NUS Research Scholarship, the Institute for Health Innovation & Technology, the Ministry of Education, the National Research Foundation, and the National Medical Research Council.

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