

Perspective

BioFET Platforms Combined with PNA Probe for MicroRNA Detection: Current Trends and Future Perspectives

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Abstract

MicroRNAs (miRNAs) represent highly promising biomarkers for non-invasive clinical diagnostics. To overcome the limitations of conventional methods, Field-Effect Transistor biosensors (bioFETs) emerge as compelling alternatives by providing label-free and real-time readouts. However, bioFETs employing canonical DNA/RNA probes face fundamental limitations due to the Debye screening effect. Peptide Nucleic Acid (PNA), a synthetic nucleic acid analog featuring an uncharged peptide-like backbone, offers an effective solution. PNA's electroneutrality allows for efficient hybridization at a low ionic strength, thereby mitigating Debye screening constraints. PNA also offers additional key advantages, including high specificity and enzymatic resistance. This work examines recent advancements in PNA–bioFETs, highlighting their potential for the development of robust platforms for clinical miRNA evaluation. Surface Plasmon Resonance (SPR) and Atomic Force Spectroscopy (AFS) are valuable tools to preliminarily characterize probe–target interactions. Key approaches, focused on implementing alternative PNA probe designs and optimizing Self-Assembled Monolayer (SAM) organization, are outlined. By presenting a cross-study comparison that correlates each strategy with the analytical outcomes of a custom-designed bioFET platform targeting miRNA 155, complemented by an overview of PNA–bioFETs across other miRNAs, we illustrate how the synergy between PNA and bioFET can approach reliable miRNA detection, paving the way for the development of Point-of-Care (POC) devices.

Keywords: Field-Effect Transistor biosensor (bioFET); microRNA (miRNA); Peptide Nucleic Acid (PNA); Self-Assembled Monolayer (SAM); Debye screening; Surface Plasmon Resonance (SPR); Atomic Force Spectroscopy (AFS)

1. Introduction

MicroRNAs (miRNAs) are short (~19–25 nt), endogenous non-coding RNAs that regulate post-transcriptional gene expression [1], and their altered expression is tightly linked to the onset and progression of various diseases, including solid and hematological malignancies [2]. Beyond their high diagnostic value, miRNAs exhibit remarkable biochemical stability within accessible biofluids, such as blood, urine, and saliva [3,4]. Such resistance supports non-invasive clinical sampling and facilitates continuous patient monitoring. The reliable quantification of miRNAs poses important analytical challenges due to their short sequence length, high sequence homology within families, and low physiological abundance [5]. Current gold methods, such as reverse transcription quantitative polymerase chain reaction (RT-qPCR) and next-generation sequencing (NGS), ensure high analytical



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performance, but they strictly rely on complex enzymatic amplification or labeling protocols to overcome miRNA-related constraints [6]. Consequently, these methods require laborious, costly, and time-consuming workflows dependent on specialized laboratory infrastructure and highly trained personnel, restricting their wider adoption in routine clinical practice [7]. To overcome these limitations, alternative biosensing approaches, ranging from optical and mechanical methods [8,9] to single-molecule electrical sensing, such as nanopore-based technology [10], have gained significant interest for amplification-free nucleic acid detection.

Alongside these strategies, electrochemical biosensors and, in particular, Field-Effect Transistor biosensors (bioFETs) have emerged as highly promising platforms for the detection of clinically relevant miRNAs, providing a powerful alternative to conventional methods [11]. By coupling a selective biorecognition element (i.e., probe) with a Field-Effect architecture (i.e., transducer), bioFETs can directly quantify miRNA concentration through electrical signal variations induced by its selective capture at the sensing interface [12]. This simple yet powerful transduction mechanism can combine high analytical performance with label- and enzymatic amplification-free operation and real-time readouts [13]. Furthermore, the compatibility of bioFETs with scalable microfabrication techniques allows for their mass production, miniaturization, and easy integration into portable, low-cost diagnostic architectures optimized for automated and decentralized testing [14].

To fully exploit bioFET's operational advantages, the features and optimization of the capturing interface are essential. Within this framework, the choice and structural design of the probe regulate device selectivity, sensitivity, and reproducibility [15]. Conventionally, antisense DNA/RNA probes are selected due to their high affinity [16]. However, their use in bioFETs introduces a fundamental limitation related to the ionic strength of the analytical environment. On the one hand, efficient hybridization between the canonical probe and target miRNA requires a relatively high ionic strength to screen the negative charges of their sugar-phosphate backbones [17]. On the other hand, a high ionic concentration triggers the Debye screening, a phenomenon where mobile counter-ions reorganize at the interface and shield target charges [18]. This limitation is particularly pronounced when approaching detection in physiological samples, which possess high native ionic strengths [19].

Peptide Nucleic Acids (PNAs) provide an effective alternative to resolve this analytical bottleneck. PNAs are synthetic nucleic acid analogs featuring a modified peptide-like backbone while maintaining the same nucleotides of their natural counterparts for selective hybridization with complementary sequence [20]. Crucially, the electroneutrality of PNA can mitigate the need for a high ionic strength during target capture [21]. This allows clinical samples to be diluted without compromising hybridization efficiency, effectively reducing Debye screening while suppressing matrix interferences. Additionally, PNA's biochemistry offers further advantages [22], including high binding affinity, remarkable sequence specificity, and resistance to enzymatic degradation, contributing to its widespread use across different biotechnological and biomedical applications [23]. Accordingly, PNA is a promising probe to fully exploit the analytical and operational benefits of bioFETs.

For an efficient recognition capability of bioFETs, PNA should be efficiently immobilized through the formation of a Self-Assembled Monolayer (SAM) [24], the overall capturing entity assembled on the transducer surface. This requires the control of probe density and other parameters to guide probe orientation, conformational freedom, and steric hindrance [25,26].

Here, the recent advancements and perspectives for bioFET platforms implemented with a PNA probe for miRNA detection have been outlined. The connection between bioFET's analytical performance and capturing interface assembly has been examined, detailing how the implementation of non-canonical PNA probe designs and optimization

of the resulting SAMs can achieve efficient miRNA biosensing. To highlight the effectiveness of the discussed strategies, a consistent cross-study evaluation centered on a custom-designed bioFET targeting miRNA 155 (miR-155) has been presented. Additionally, the analytical performances of different PNA-bioFET platforms targeting other clinically relevant miRNAs have been overviewed.

2. Insights into BioFET-Based Detection

2.1. BioFET Operating Principle

A conventional Field-Effect Transistor (FET) is a three-terminal electronic device where the charge carrier density, and consequently, the electrical conductivity, of a semiconductor channel connecting the source and drain electrodes is modulated by an external potential (V_G), which is applied to the gate electrode under a fixed drain–source potential (V_{DS}) [27]. In a bioFET platform, the gate surface is modified by the immobilization of a selective biorecognition element (i.e., probe) [28]. The specific binding between the immobilized probe and intrinsically charged analyte introduces a perturbation in the surface charge density, which modifies V_G and then the source–drain current (I_{DS}). This potentiometric transduction mechanism can be described in terms of the variation in the drain–source current (ΔI_{DS}) to the variation in the gate potential (ΔV_G) through the transistor transconductance (g_m) [29]:

$$\Delta I_{DS} = g_m \Delta V_G \quad (1)$$

In many cases, the modulation of ΔV_G induced by target capture can be modeled using a classical Langmuir adsorption isotherm [30]:

$$\Delta V_G = \frac{qN_R}{C} \frac{C_A}{C_A + K_D} \quad (2)$$

where q represents the effective net charge introduced per single capture event, N_R is the surface density of available receptor (i.e., probe) sites, C is the interfacial capacitance, C_A is the bulk analyte concentration, and K_D is the equilibrium dissociation constant.

In biosensing applications, two primary measurement modalities are carried out: current–voltage (I–V) characteristics and real-time I_{DS} monitoring (Figure 1) [31].

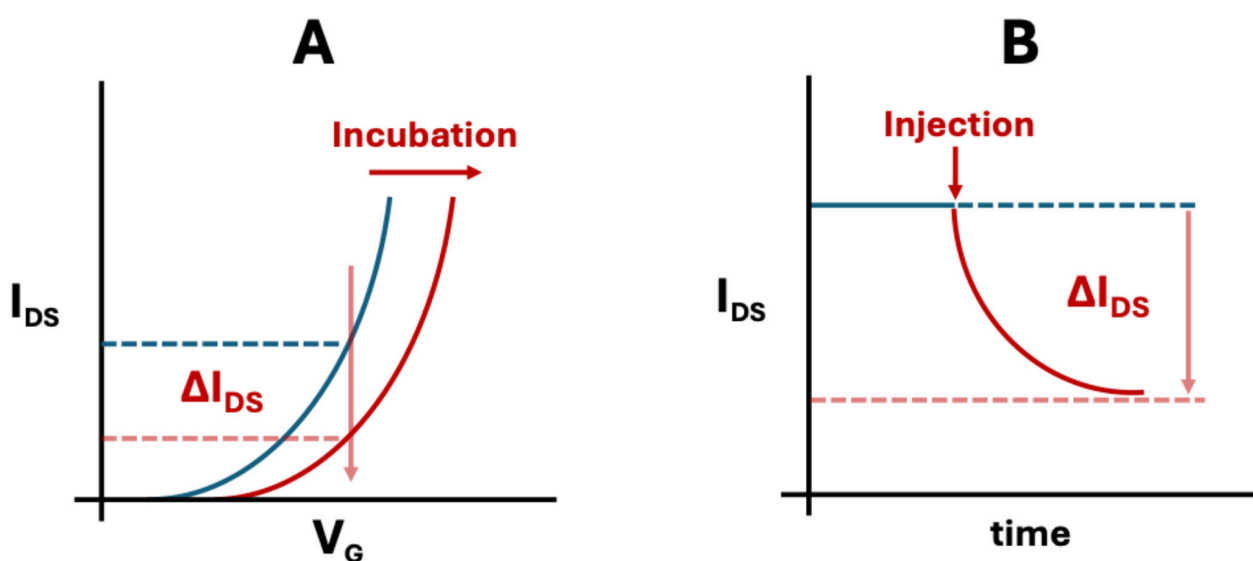


Figure 1. BioFET signal acquisition. (A) Current–voltage (I–V) characteristics before and after surface functionalization or target capture, leading to current variation (ΔI_{DS}); (B) real-time current under fixed V_G and V_{DS} , with dynamic current variation (ΔI_{DS}) over time after target injection.

The I-V characteristics are typically recorded by sweeping V_G while maintaining a constant V_{DS} . Upon probe immobilization or target capture, the accumulation of charges induces a shift in the I-V curve. This configuration is particularly suited for evaluating working electrode functionalization steps by acquiring and comparing the I-V profiles during the interface preparation [32].

Conversely, real-time monitoring is conducted by continuously tracking I_{DS} over time under fixed V_{DS} and V_G . In this modality, target capture by the probe triggers an immediate change in I_{DS} . Such continuous recording represents the optimal choice for direct analyte detection, making it highly attractive for potential clinical applications where rapid diagnostic readouts and real-time monitoring are required [17,32].

2.2. Materials and BioFET Set-Up for Biosensing Applications

Selecting the FET transducer and related semiconductor channel material represents important parameters in biosensor engineering. Metal-Oxide-Semiconductor FET (MOSFET) remains the benchmark transducer choice for biosensing applications [33]. This preference is driven by mature, industry-standard complementary MOS (CMOS) fabrication processes, which guarantee high signal-to-noise ratios combined with highly reproducible mass production.

To safely employ FET transducers in liquid environments, the architecture must prevent the potential chemical degradation and baseline drift that could occur when an electrolytic solution directly contacts the device components. To overcome this operational issue, the Extended-Gate FET (EG-FET) configuration is generally utilized [34]. As schematically illustrated in Figure 2, the sensing interface is physically isolated from the semiconductor channel and connected to the transistor's gate terminal via a passivated conductive line, with the working electrode acting as the Extended Gate.

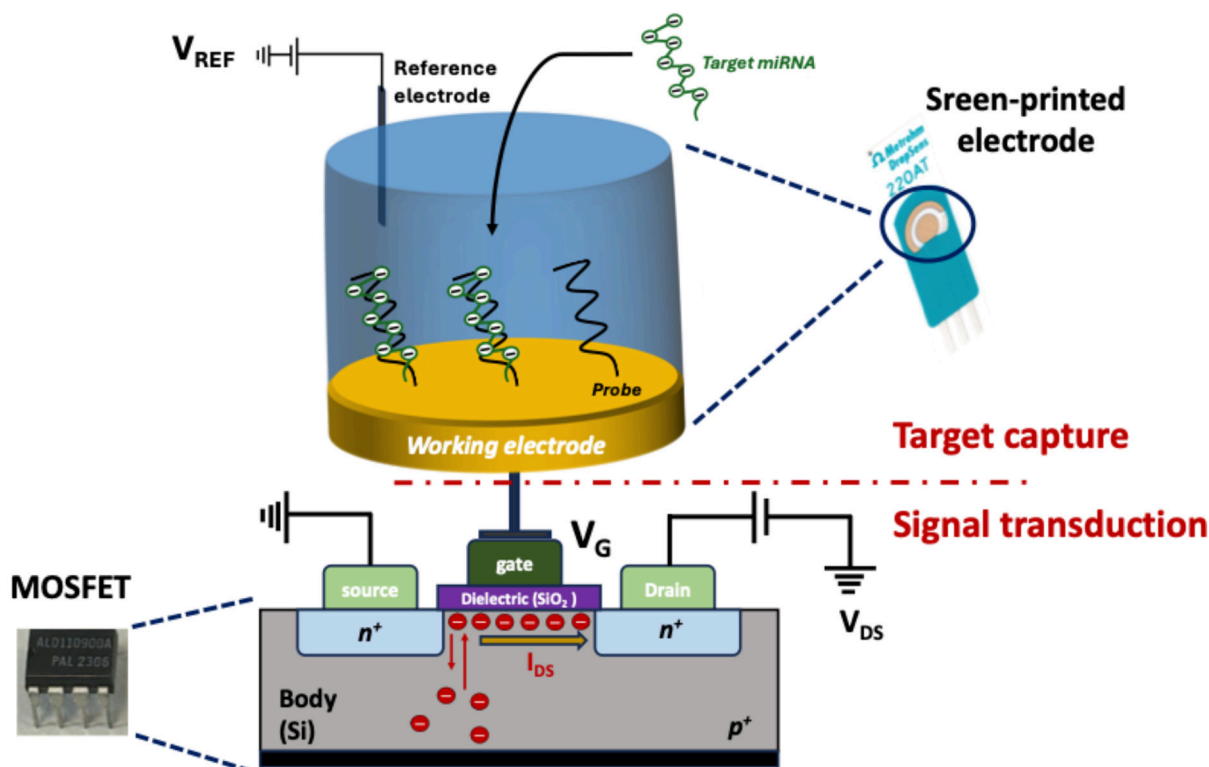


Figure 2. Representation of the EG-bioFET configuration. Target capture is occurring on a disposable, external gold Screen-Printed Electrode (SPE) providing the working electrode functionalized with probes and coupled to the gate of a Metal-Oxide-Semiconductor Field-Effect Transistor (MOSFET) for signal transduction.

This spatial decoupling protects the electronic components from electrolyte contamination, allowing the FET transducer to be integrated permanently and confining sample exposure exclusively to a low-cost, disposable sensing interface [35]. For the mass production of the working electrode, screen-printed technologies offer a highly reproducible manufacturing platform for single-use employment [36]. Within this fabrication framework, gold stands out as the ideal surface material. Indeed, gold combines high electrical conductivity and electrochemical inertness, with a well-characterized surface functionalization strategy based on the formation of covalent gold–thiol (Au–S) bonds, for the basis of widely employed applications in biosensing [37].

2.3. Ionic Strength and Debye Screening

As already mentioned, the sensitivity and operational feasibility of bioFETs are strictly limited by the ionic strength of the analytical medium. When a functionalized electrode surface is immersed in an electrolyte solution, mobile ions spontaneously reorganize in response to localized interfacial charges, giving rise to the Electrical Double Layer (EDL) (Figure 3) [38].

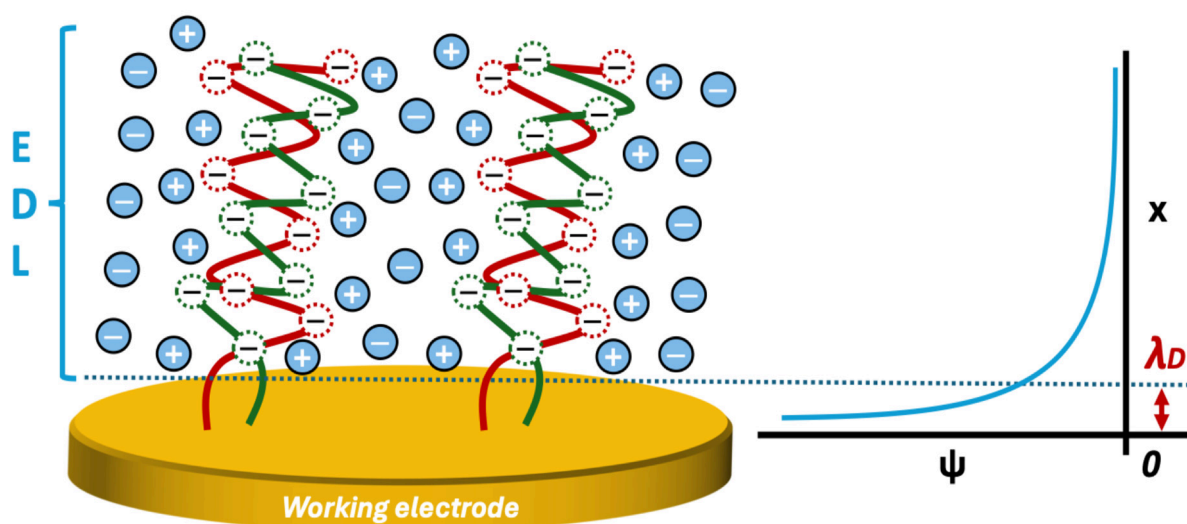


Figure 3. Physics of Debye screening. Electrical double layer (EDL) formation at the working electrode surface immersed in an electrolyte solution, leading to an exponential decay of the surface potential (ψ) characterized by the Debye length (λ_D).

Such a process can be well-described by the linearized Poisson–Boltzmann equation, from which the electrostatic potential (ψ) decays exponentially as a function of the distance (x) from the electrode surface [39]:

$$\psi = \psi_0 \left(e^{-\frac{x}{\lambda_D}} \right) \quad (3)$$

where ψ_0 is the surface potential at $x = 0$, and λ_D represents the Debye length; namely, the characteristic shielding distance over which electrostatic perturbations persist within the solution [35], as expressed by:

$$\lambda_D = \sqrt{\frac{\epsilon_0 \epsilon_r \kappa_B T}{2e^2 I}} \quad (4)$$

where ϵ_0 is the vacuum permittivity, ϵ_r is the relative permittivity of the solvent, κ_B is the Boltzmann constant, T is the absolute temperature, e is the elementary charge, and I is the ionic strength of the electrolytic solution ($I = \frac{1}{2} \sum_i n_i z_i^2$, where n_i and z_i represent the concentration and valence of each ionic species, respectively). Under physiological conditions, the ionic strength typically ranges between 150 mM and 300 mM, correspond-

ing to a Debye length compressed to sub-nanometric values [40]. Because the physical dimensions of the probe–miRNA complex exceeds the Debye length [41], the target charges are almost screened, preventing them from significantly influencing the potential at the working electrode. This spatial constraint is further accentuated since canonical nucleic acid hybridization requires moderately high salt concentrations to screen the electrostatic repulsion between interacting strands [42]. For these reasons, mitigating the Debye screening while maintaining efficient hybridization is paramount for translating bioFET platforms into viable diagnostic tools for real clinical applications.

3. PNA as a Promising Probe for miRNA Detection by BioFETs

Peptide Nucleic Acid (PNA) is a synthetic nucleic acid mimic in which the conventional, negatively charged sugar–phosphate backbone of natural DNA/RNA is replaced by an uncharged, achiral backbone comprising repeating *N*-(2-aminoethyl) glycine units [20]. Despite this modification, as illustrated in Figure 4, PNA maintains the canonical nucleotides of natural nucleic acids, which are bound to the peptide-like backbone via *methylene carbonyl* bonds [20].

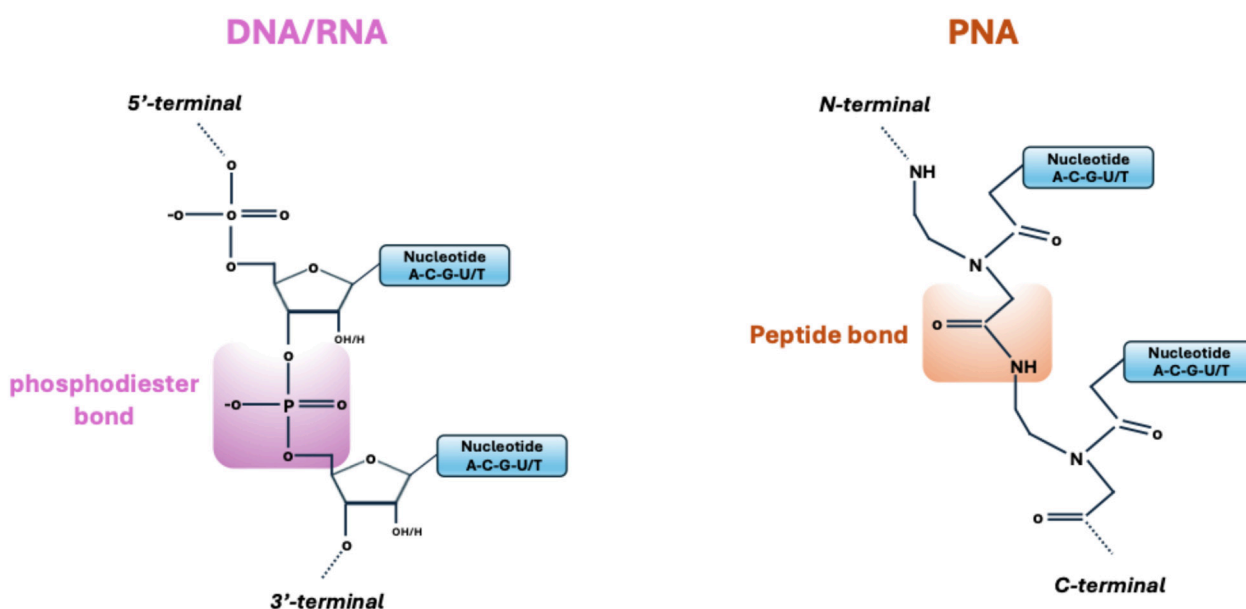


Figure 4. Comparison of RNA/DNA and PNA. RNA/DNA features a negatively charged sugar–phosphate backbone based on phosphodiester bonds, and PNA exhibits a neutral *N*-(2-aminoethyl) glycine backbone based on peptide bonds, with both structures maintaining the same canonical nucleotides for selective hybridization.

This architecture preserves the same rules that mediate the selective formation of canonical duplexes, allowing PNA to hybridize with complementary DNA/RNA strands via standard Watson–Crick base pairing. PNA’s electroneutrality has the direct consequence of reducing the electrostatic repulsion between hybridizing partners, conferring a higher intrinsic binding affinity to PNA compared to its canonical counterpart [43]. Such improved affinity is closely correlated with the remarkable sequence discrimination capability of PNA [44]. Indeed, even few-base mismatch destabilizes the duplex formation, thus limiting non-specific interactions. This feature is particularly important when approaching the detection of target sequences characterized by high homology, like miRNAs.

The reduced electrostatic repulsion between partners provides decisive operational advantages for the bioFET detection of miRNAs. By mitigating the need for counterion attenuation, PNAs also maintain a high affinity for target sequences, even at a low

ionic strength [45]. This capability can circumvent the limitations of Debye screening in potentiometric sensing of miRNAs, as operating at lower ionic strength increases the Debye length (λ_D) and allows for an effective modulation of working electrode surface potential by the captured target charges [46]. Importantly, sample dilution is made viable by the high affinity of PNAs, which guarantees efficient target capture even when low-abundance miRNA biomarkers are diluted. This bypasses complex, time-consuming pre-analytical workflows while mitigating matrix interferences from background biological components.

A further advantage of PNA is the maximization of the potentiometric signal [47]. In bioFETs implemented with traditional, negatively charged antisense probes, hybridization induces a transition from an already charged layer to a more densely charged layer, typically yielding a poor signal-to-noise ratio and low analytical resolution. Conversely, a PNA-functionalized interface undergoes a net transition from an uncharged layer to a charged layer upon miRNA capture, generating a better-resolved analytical signal.

Finally, because the PNA backbone lacks natural phosphodiester bonds and adopts a non-canonical structural conformation, both the PNA molecules and the corresponding heteroduplexes can escape from enzymatic degradation [48], maintaining structural integrity during prolonged exposure to complex biological samples where natural oligonucleotides would otherwise undergo rapid degradation.

In addition to these benefits, some limitations of PNA probes should be considered. Due to their uncharged backbone, PNAs exhibit a propensity for self-aggregation [49]. This issue can be minimized through thermal pre-denaturation treatments before surface immobilization [50]. The chemical synthesis of PNA remains more expensive than standard oligonucleotide production [51]. However, the initial expense is well-balanced by the compelling advantages that PNA offers over canonical RNA/DNA probes, as previously discussed.

4. Recent Implementation Strategies for PNA–bioFETs Devoted to MiRNA Detection

4.1. SPR and AFS as Predictive Tools to Evaluate PNA/miRNA Hybridization on Surface

The quantitative characterization of the interactions between an immobilized capture probe and target in solution represents a fundamental step to evaluate interfacial binding efficiency, which is crucial in determining the analytical performance of any biosensor architecture. In this respect, surface-sensitive analytical techniques like Surface Plasmon Resonance (SPR) and Atomic Force Spectroscopy (AFS) can provide valuable preliminary investigations. These methodologies, by approximating the conditions characterizing the biosensor capturing interface, offer powerful complementary approaches at bulk (SPR) and single-molecule (AFS) levels [52,53].

SPR permits real-time monitoring of the molecular association and dissociation processes taking place directly at a functionalized substrate (Figure 5A, top). By tracking changes in resonance units (RU) upon molecular binding (for more details, see Ref. [52]), this technique allows for the extraction of association rate constant (k_{on}), dissociation rate constant (k_{off}), and equilibrium dissociation constant ($K_D = k_{off}/k_{on}$) related to the affinity.

As an example, an SPR sensorgram obtained by exposing an immobilized PNA probe to increasing concentrations of miRNA 155 (miR-155) while monitoring the RU profile over time is shown in the bottom of Figure 5A [54]. The sharp rise in RU reveals a rapid target capture during the association phase, whereas the subsequent dissociation phase shows a negligible signal decrease. The 1:1 Langmuir adsorption model best describes the kinetics of the hybridization process, yielding a k_{on} of $\sim 3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and a k_{off} of $\sim 2 \times 10^{-5} \text{ s}^{-1}$ [54]. The order of magnitude of these parameters carries direct operational implications for PNA-based biosensors, especially for bioFET platforms devoted to miRNA detection. The k_{on} value denotes the fast association of partners, matching the operational demands for rapid readouts, while the

k_{off} value implies that once the target miR-155 is captured, the resulting heteroduplex remains stably bound, ensuring a sustained charge perturbation at the bioFET working electrode. The resulting K_D of $\sim 0.5 \times 10^{-9}$ M is rather low, allowing for an efficient detection of low-abundance biomarkers, like miRNAs.

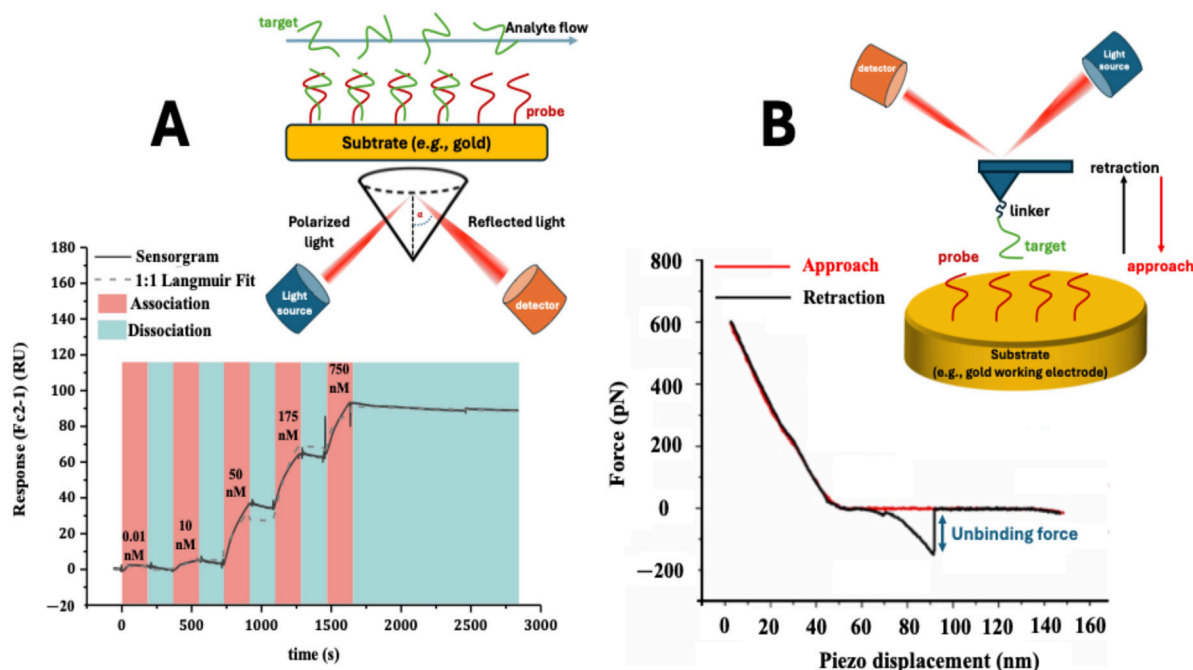


Figure 5. PNA/miR-155 hybridization by SPR and AFS technique. (A) Schematic illustration of the SPR method (**top**) and a representative sensorgram upon adding increasing concentrations of miR-155 onto a PNA-modified gold substrate (**bottom**), with the data fitted using the 1:1 Langmuir model (adapted from Ref. [54]); (B) schematic illustration of the AFS method (**top**) and a representative approach–retraction force curve with a miR-155-modified cantilever and a PNA-functionalized gold working electrode (**bottom**)—the unbinding force determination is highlighted (adapted from Ref. [55]).

To complement SPR bulk data, AFS can be employed to investigate hybridization kinetics at the single-molecule level. AFS operates as a highly sensitive technique, where the target molecule is covalently immobilized on a tip at the end of a cantilever through a flexible cross-linker, while the probe is immobilized on a substrate (Figure 5B, top). Force curves are acquired by cyclically approaching and retracting the cantilever, at different loading rates (LRs, given by dF/dt), towards and away from the substrate to promote the formation of the probe–target complex and its subsequent unbinding (for more details, see Ref. [53]). An analysis of the force required for the unbinding of the formed complex allows for the extraction of k_{off} and the width of the energy barrier (x_β). A representative force curve derived from an approach–retraction cycle involving the PNA probe and miR-155 target, with the first immobilized on the substrate and the second on the cantilever tip, is shown in bottom of Figure 5B [55]. AFS revealed a k_{off} of $\sim 4 \times 10^{-4} \text{ s}^{-1}$, consistent with that obtained via SPR, and a x_β value of $\sim 0.7 \text{ nm}$, which further underscores the stability of the PNA/miR-155 complex. Subsequent AFS investigations across a wide ionic strength range (50–300 mM) demonstrated that the binding energy landscape of PNA/miR-155 hybridization was slightly affected by the ionic strength [55]. This provides a solid ground for operating bioFETs in low-salinity detection media without compromising the fundamental recognition kinetics of the surface-bound PNA capture probe.

Overall, the presented analyses prove that the synergistic application of SPR and AFS can serve as a valuable predictive approach to systematically evaluate and guide the integration of PNA probes into bioFET platforms for high-performance biosensing.

4.2. Controlling PNA Probe Density for the Formation of Highly Reactive SAMs

The intrinsic affinity of the PNA probe constitutes the molecular foundation of efficient miRNA recognition; however, the overall analytical performance of bioFET platforms is influenced by the organization of capturing Self-Assembled Monolayers (SAMs) at the working electrode surface. Among different structural parameters like phase homogeneity, thickness, and surface coverage [56], probe density, related to the spacing between adjacent probes, strongly affects the capability of PNA to efficiently capture the target miRNA and is regarded as one of the most critical parameters [57].

At a low probe density, SAMs are generally characterized by PNA probes as not sufficiently packed to ensure their correct orientation for miRNA recognition (Figure 6A) [58]. Conversely, a high probe density promotes the formation of SAMs in which PNA probes are in proximity, thus generating the occurrence of the steric hindrance problem during miRNA capture (Figure 6B) [59].

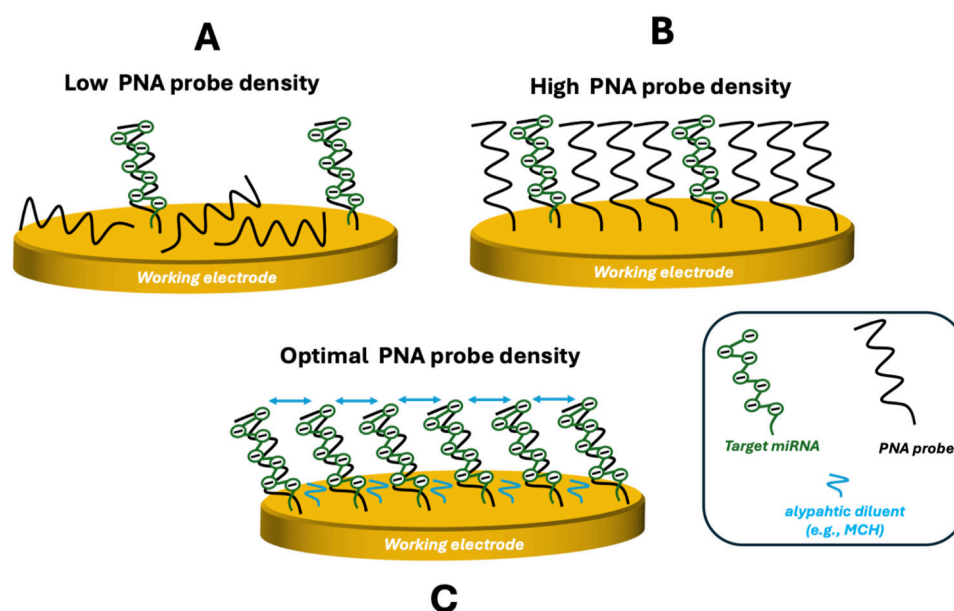


Figure 6. PNA probe density's influence on SAM organization at the working electrode. (A) Low probe density, (B) high probe density, and (C) optimal probe density achieved by introducing an aliphatic diluent (e.g., MCH).

The use of small diluent molecules to be simultaneously co-immobilized with PNA represents an effective strategy to ensure the formation of responsive SAMs [60,61]. These molecules, by occupying a controlled fraction of the available surface sites, modulate probe density and inter-probe spacing, improving hybridization efficiency (Figure 6C). A collection of diluent-based approaches has been investigated with different electrochemical architectures, using several molecule types and protocols [62,63]. In most cases, such diluents are aliphatic or aromatic thiols with a variable number of carbon atoms [64]. For the optimization of SAMs characterized by short probe sequences (like PNA for miRNA capture), 3–6 carbon unit aliphatic chains are generally employed, and 6-mercapto-1-hexanol (MCH) is one of the most widely used [65]. To fully exploit the potential of MCH in controlling probe density, different aspects regarding the co-immobilization protocol (such as the incubation time and temperature or the ionic strength and pH of the immobilization buffer) must be accurately evaluated [61]. Alongside these parameters, probe concentration and

its ratio relative to MCH are crucial to maximize PNA's capability to capture miRNAs [61]. Electrochemical Impedance Spectroscopy (EIS) studies have shown that the introduction of MCH co-immobilized at an intermediate ratio for probe dilution, leading to an optimized SAM organization with an enhanced hybridization efficiency [66]. Such results highlight the beneficial effect of a diluent-based approach to guide SAM formation, which can also be exploited in the context of bioFET-based detection.

4.3. Tuning PNA Probe Design for Enhanced BioFET Responses

The miRNA capture by the PNA probe generally promotes the formation of vertically oriented heteroduplexes [67]; consequently, only a fraction of the target charges is close to the working electrode surface (Figure 7A).

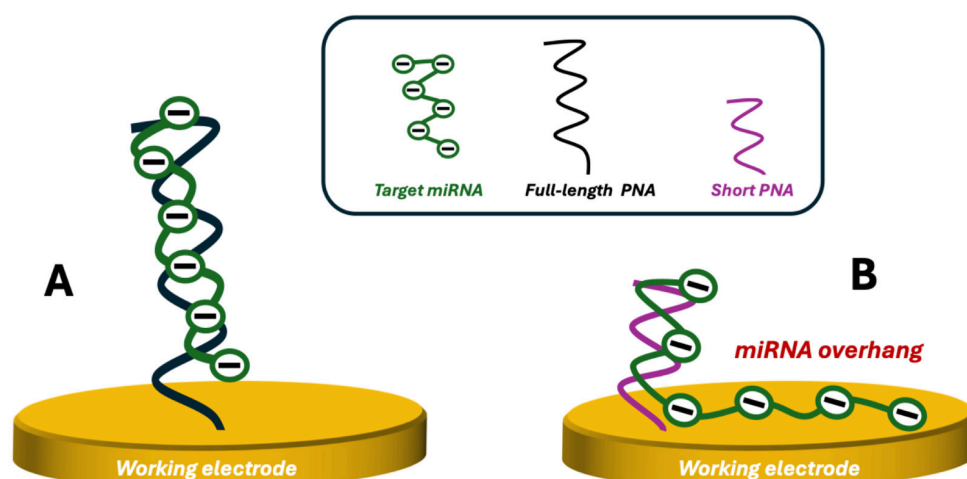


Figure 7. Comparison of FL-PNA and S-PNA resulting heteroduplexes. (A) FL-PNA/miRNA hybridization resulting in vertically oriented heteroduplex; (B) S-PNA/miRNA hybridization resulting in a heteroduplex, with an unhybridized miRNA portion (i.e., overhang) bent close to working electrode surface.

This aspect, combined with the relatively low number of miRNA charges, limits signal transduction despite efficient hybridization and Debye screening attenuation. Therefore, maximizing bioFET sensitivity requires addressing how target charges are spatially distributed across the working electrode surface. In this context, different approaches involving the introduction of alternative PNA design have been evaluated. Some strategies involve integrating multiple thiol anchoring points at different positions along the PNA backbone, forcing the resulting heteroduplex to align horizontally rather than vertically on the surface [67]. Other approaches deploy hairpin-like PNA probes that undergo a significant conformational change upon hybridization, mechanically pulling the target downward closer to the surface [68].

Alternatively, manipulating the probe length offers a straightforward strategy for target charge relocation by using a short PNA probe (S-PNA) [69,70]. Shortening the PNA sequence compared to miRNA results in unhybridized single-stranded segments, termed overhangs. Because these overhangs are not involved in hybridization, they retain enough flexibility to bend toward the surface, concentrating more miRNA charges near the working electrode (Figure 7B). The effectiveness of the overhang strategy has been recently demonstrated by EIS analyses using a 10-mer short PNA (S-PNA) probe, combined with a co-immobilization protocol involving MCH, to create overhangs upon hybridization with miR-155 [71]. The use of S-PNA enhanced signal generation upon miR-155 capture, proving that the resulting overhangs could amplify the electrochemical transduction, even when implemented in bioFET frameworks.

4.4. Analytical Performance Achieved by a Recent PNA–bioFET Platform for miRNAs

The implementation of PNA probe into bioFET, along with various strategies, can achieve high-performance biosensing of clinically relevant miRNAs. Figure 8 summarizes the analytical performance achieved by a custom-designed EG-bioFET platform (for more detail, see Section 2.2 and Refs. [54,66,71]) devoted to the detection of miR-155, a clinically relevant representative miRNA [72]. Evaluating different PNA-based approaches from a unified perspective highlights the effectiveness of each strategy with respect to the analytical performance of the bioFET platform. Although based on independent studies with different concentration ranges and small experimental variations, these analyses share a common bioFET platform with identical operating conditions. Moreover, the consistent LoD calculation, obtained by $3\sigma/s$, where σ is the standard deviation of the blank signal and s is the slope of the calibration plot, allows a meaningful comparison between the different results.

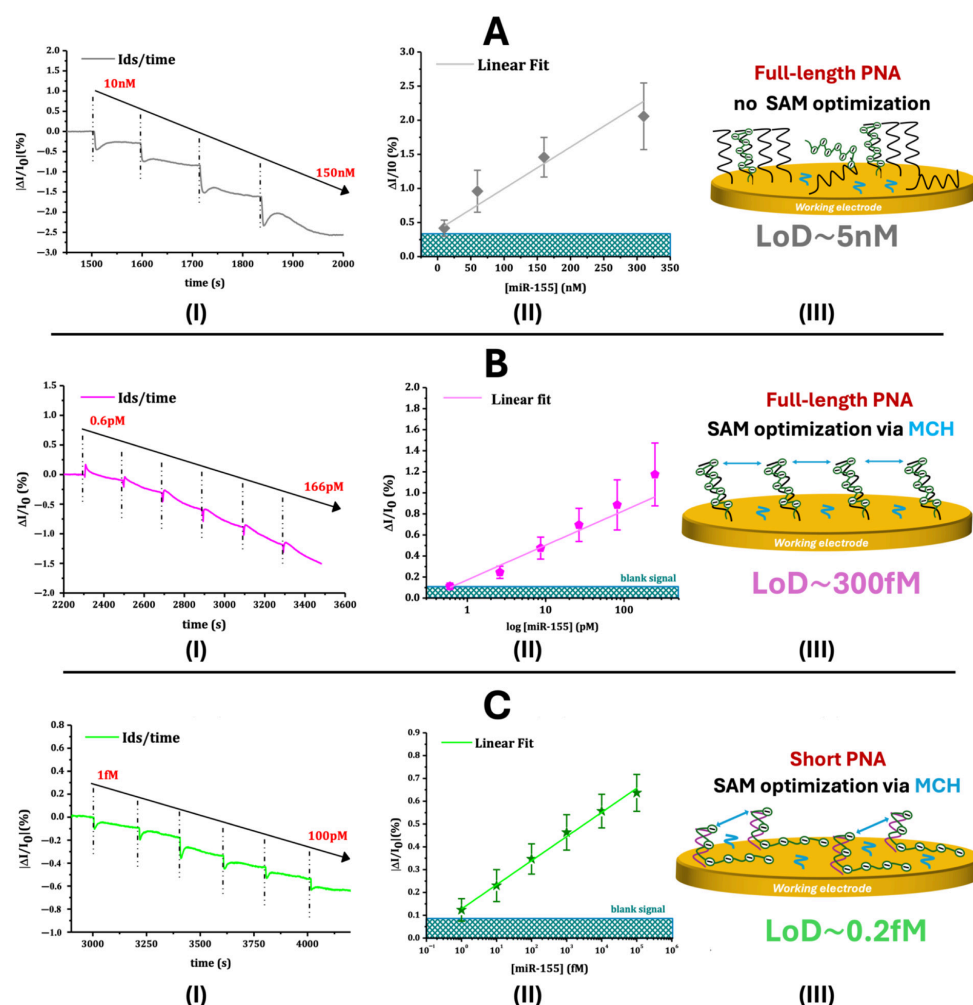


Figure 8. Analytical performance of an EG-bioFET with different PNA-based strategies. (A) FL-PNA probe—the real-time response across target injections from 10 to 150 nM (I), the corresponding calibration plot (II), and the schematic illustration of the relating SAM (III) (adapted from Ref. [54]); (B) FL-PNA probe optimized via MCH co-immobilization—the real-time response across target injections from 0.6 and 166 pM (I), the corresponding linear calibration plot (II), and the schematic illustration of the relating SAM (III) showing the correct probe spacing coordinated by MCH (adapted from Ref. [66]); (C) S-PNA probe optimized via MCH co-immobilization—the real-time response across target injections from 1 fM to 100 pM (I), the corresponding calibration plot (II), and the schematic illustration of the relating SAM (III) showing S-PNA/miRNA heteroduplex arrangement within the well-organized SAM (adapted from Ref. [71]).

A real-time biosensing experiment performed by exposing a PNA probe, immobilized on the working electrode without any optimization of the SAM organization, to increasing miR-155 concentrations within a nanomolar range (10 to 150 nM) is shown in Figure 8 [54]. The sequential additions of the target miR-155 induce a prompt modulation of the source-drain current (I_{DS}) under fixed electrical potentials. This direct electrostatic transduction reflects the PNA/miR-155 hybridization behavior predicted by SPR and AFS, allowing for the occurrence of fast and stable capture events at the working electrode surface. The corresponding calibration plot displays a concentration-dependent linear trend (Figure 8(AII)), from which a limit of detection (LoD) of ~5 nM was derived [54]. However, since this biosensing experiment was performed without fine-tuning the PNA probe density, this could have led to unoptimized SAMs with reduced capturing efficiency (Figure 8(AIII)).

A real-time biosensing experiment obtained through the implementation of a co-immobilization strategy involving MCH for PNA probe density optimization, and in a lower miR-155 concentration range (0.6 to 166 pM), is shown in Figure 8(BI) [66]. The effectiveness of the MCH co-immobilization approach was provided by the corresponding calibration plot (Figure 8(BII)). Compared to the earlier study [48], under similar operating conditions, the platform shows a remarkable 1000-fold improvement in sensitivity, yielding an LoD of ~300 fM [66]. This enhancement can be ascribed to the formation of a well-organized SAM, which should be sufficiently spaced to prevent steric hindrance occurrence yet dense enough to ensure the correct probe orientation for maximized hybridization efficiency (Figure 8(BIII)).

To further improve bioFET responses, an alternative PNA probe design can be introduced to force more target charges close to the working electrode surface. A real-time biosensing experiment was performed by introducing a 10-mer S-PNA probe design, along with a co-immobilization approach involving MCH and with miR-155 additions further lowered to femtomolar range (1 fM to 100 pM), as shown in Figure 8(CI) [71]. Under these conditions, the bioFET platform maintained remarkable detection capability even at the lowest analyzed concentrations; from the derived calibration plot (Figure 8(CII)), an LoD of ~0.2 fM was extracted [71]. This performance reflects a highly favorable capturing interface where the optimal probe density, achieved through an MCH co-immobilization protocol tailor-made for the S-PNA probe, works in synergy with the shortened design (Figure 8(CIII)). Upon target hybridization, this sequence reduction yields unhybridized overhangs that effectively relocate a higher density of target charges close to the working electrode surface, resulting in a significant analytical improvement compared to canonical FL-PNA probe evaluated in previously discussed works [54,66].

Crucially, across different implementations, the platform exhibited stable baseline currents (0.01 μ A over 200 s) and signal reproducibility (RSD < 5%), confirming that the achieved analytical sensitivity is supported by solid operational stability and measurement reliability.

The presented analytical framework shows the great potential of PNA probes in bioFET-based detection of miRNAs. The achievement of analytical sensitivity suitable for miRNA-based diagnostics requires the refinement of key aspects related to probe designs and the resulting capturing SAMs to extend the potential clinical applicability of bioFETs.

Approaching miRNA Detection by PNA–bioFETs in a Complex Biological Matrix

The signal obtained by a bioFET platform functionalized with a 10-mer S-PNA probe and exposed to diluted (1:100) human serum spiked with three different miR-155 concentrations (10 fM, 10 pM, and 10 nM) is shown in Figure 9A.

All tested miR-155 concentrations, covering the physio-pathological range typically observed for miRNAs [73], induced a higher current variation compared to the blank control (1:100 diluted serum without miR-155). These results, combined with the extracted

recovery rate (RR) and relative standard deviation (RSD) falling within ranges suitable for clinical applications, confirm the feasibility of robust miRNA detection in a complex biological matrix.

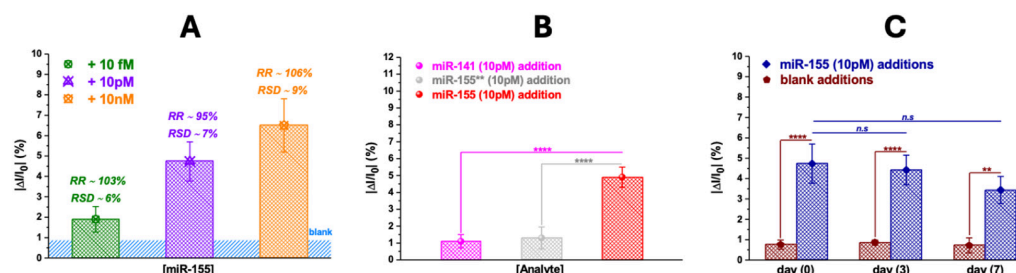


Figure 9. Analytical performances of an EG-bioFET in diluted human serum. (A) bioFET responses to diluted (1:100) human serum samples spiked with three miR-155 concentrations (10 fM, 10 pM, and 10 nM); (B) bioFET response to diluted (1:100) human serum samples spiked with the target miR-155 (10 pM), non-complementary miR-141 (10 pM), and miR-155** double mismatch isoform (10 pM); (C) bioFET responses to repeated exposure to diluted (1:100) human serum with or without miR-155 over a 7-day period. Significance levels based on an unpaired *t*-test: (n.s., $p > 0.5$), ($** p < 0.01$), ($*** p < 0.0001$). (adapted from Ref. [71]).

Specificity, a critical requirement when targeting short sequences with high sequence homology like miRNAs, was evaluated by exposing the S-PNA–bioFET platform to 1:100 diluted human serum spiked with the target miR-155 (10 pM), a non-complementary miRNA 141 (miR-141) (10 pM), and miR-155** double-base mismatch (10 pM), as shown in Figure 9B. The platform exhibited remarkable specificity, as the S-PNA probe was able to efficiently discriminate both the non-complementary and double-mismatch variants, despite the reduced number of bases available for hybridization.

For future clinical translation, the long-term stability of the platform represents an additional key aspect, as the capability to store the functionalized device can significantly influence its shelf-life and viability for realistic clinical applications. The S-PNA–bioFET responses to miR-155 exposure (10 pM) after 0, 3, and 7 days of storage are shown in Figure 9C. The platform maintained consistent responses, thus confirming that storage and repeated exposure to a complex matrix do not compromise its detection efficiency.

Overall, these analytical achievements, obtained in a complex matrix with no pre-treatments other than a simple dilution, demonstrate that the combination of a custom-designed bioFET platform (comprising inexpensive, mass-produced components), an optimized PNA probe design, and a controlled SAM structural organization can approach the real-time, label-free, and enzymatic amplification-free detection of miRNAs directly in complex biological samples. Nevertheless, while these results in spiked serum provide a solid analytical foundation, clinical translation would require validation on real patient cohorts to quantify endogenous miRNAs, as well as direct benchmarking against standard methods like RT-qPCR.

4.5. Other PNA–bioFETs for miRNA Detection

Several research groups have implemented PNA into different bioFET platforms to target a variety of clinically relevant miRNAs. For instance, Gao et al. employed a PNA-functionalized silicon-based FET to achieve the reliable detection of miRNA-3646, miRNA-4484, and miRNA-k12-5-5p [46]. The use of PNA probes was pivotal in suppressing charge-screening constraints and minimizing non-specific interactions, thereby yielding sub-picomolar LoD for the three miRNAs and good specificity in serum samples. Similarly, Yu et al. developed an integrated FET-sensing microsystem utilizing PNA capturing layers for the detection of exosomal miR-10b [74]. The device demonstrated remarkable analytical

performance, achieving target discrimination against mismatch isoforms and detection at the sub-femtomolar level in exosome extracts. Alternative bioFET architectures have also exploited PNA probes to enhance sensing robustness. Al Ghamdi et al. reported a PNA-functionalized heterojunction thin-film FET designed as a scalable and reusable platform for monitoring miR-141-5p [75]. The platform achieved single-nucleotide discrimination accompanied by an LoD of 0.6 fM. Furthermore, straightforward surface regeneration allowed for repeated device reuse without compromising sensing performance. By integrating a high-throughput and array-based detection, Ganguli et al. incorporated PNA probes onto a large-scale ISFET array platform [76]. The PNA implementation provided robust detection of miR-Let-7b with a broad dynamic range and low LoD. Collectively, these studies reinforce that PNA probes represent a highly adaptable approach. Across different FET configurations, the use of PNA yields low femtomolar to sub-picomolar LoDs for different target miRNAs, remarkable discrimination capability, and high operational feasibility in real or bio-like matrices.

5. Conclusions and Perspectives

In this work, we have reviewed and discussed the recent advancements in integrating PNA probes into bioFET platforms for the detection of clinically relevant miRNAs. While bioFETs inherently offer remarkable operational benefits over standard molecular methods, platforms employing canonical antisense DNA/RNA probes are fundamentally limited by the Debye screening effect. The use of electrically neutral synthetic PNA represents an effective solution, ensuring efficient miRNA target capture at low ionic strengths. Moreover, PNA's biochemistry provides additional key advantages particularly suited for clinical applications. The adoption of SPR and AFS techniques can provide key insights to preliminarily evaluate the probe–target complex formation on surfaces. Fine-tuning of the capturing interface has been demonstrated to be paramount; the introduction of an alternative design with the concomitant optimization of the PNA probe density and the resulting SAMs by diluent-based approaches can improve device performance without introducing any complex operations. Analytical validation of these strategies in complex matrices has confirmed PNA–bioFETs as a promising technology able to overcome the drawbacks of conventional detection methods. Looking forward to achieving full clinical translation, some critical challenges should be addressed. Since complex pathologies, like tumors, rarely rely on a single deregulated miRNA, future perspectives must orient toward implementing electrodes featuring multiple independent working surfaces for the simultaneous detection of multiple selected miRNAs. Furthermore, analytical testing must expand to real patient cohorts to validate the robustness of PNA-assisted sample dilution. Finally, efforts should converge toward automated detection processes and component miniaturization to successfully translate these platforms into Point-of-Care (PoC) devices. In conclusion, consolidating the discussed PNA–bioFET implementation strategies, combined with the evolution toward automated and multi-target systems validated in real patient samples, will drive the development of low-cost, decentralized diagnostic tools capable of fully exploiting the clinical potential of miRNA biomarkers.

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