The Super-Sleuth Sensor: Using DNA Origami and Gold Nanoparticles to Find miRNA21

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Abstract: This study presents the development of a novel biosensing platform based on DNA tetrahedron–gold nanoparticle (AuNP) complexes for the ultrasensitive detection of miRNA21, a critical cancer biomarker. Through the precise self-assembly of thiol-modified DNA strands, stable three-dimensional nanostructures were constructed on AuNP surfaces. The platform incorporates a hybridization chain reaction (HCR) mechanism to enable effective signal amplification, thereby achieving exceptional detection sensitivity. Under optimized conditions, the biosensor demonstrated a broad linear detection range from 100 fM to 100 pM, with a detection limit as low as 1 fM for mimicDNA21. Moreover, it exhibited high specificity and stability against interfering molecules, highlighting its significant potential for use in clinical diagnostics and point-of-care testing applications. The successful integration of the structural precision of DNA nanotechnology with the enzymatic-free amplification power of HCR presents a generic and robust sensing strategy. This platform can be readily adapted for the detection of other disease-related biomarkers by simply reprogramming the probe sequences. Furthermore, by implementing a step-wise assembly strategy and rigorous buffer optimization, this study addresses key challenges in coordinating multi-component self-assembly and minimizing non-specific interactions. Consequently, this work not only establishes a high-performance sensor for miRNA21 but also provides a versatile methodological framework for constructing next-generation DNA-based biosensors.

1. Introduction

MicroRNA21 (miRNA21) has emerged as a crucial biomarker in oncology, with demonstrated overexpression in various cancers, including breast, lung, and colorectal cancers [1]. Its detection holds substantial promise for early cancer diagnosis and therapeutic monitoring. However, conventional detection methods such as quantitative real-time PCR and Northern blotting face limitations including requiring sophisticated thermal cycling equipment, lengthy procedures, and exhibiting only moderate sensitivity, which restricts their application in resource-limited settings. Furthermore, they encounter significant challenges in directly and accurately analyzing complex biological matrices such as serum, where components like nucleases and proteins can lead to false results. As an oncogenic miRNA, miRNA21 functions by repressing the expression of pivotal tumor suppressor genes, thereby promoting cell proliferation, invasion, and metastasis. Its reliable quantification in bodily fluids like serum or plasma is therefore of paramount importance for liquid biopsy applications.

DNA nanotechnology offers innovative solutions to these challenges [2]. DNA tetrahedrons, formed through self-assembly of four specifically designed DNA strands [3], present unique advantages for biosensing applications. Their rigid three-dimensional structure provides exceptional stability against nuclease degradation, precise spatial control over probe positioning, and facile functionalization through thiol groups for surface anchoring. When combined with gold nanoparticles' exceptional optical properties [4] and the powerful signal amplification capability of hybridization chain reaction [5], these nanostructures create an ideal platform for ultrasensitive molecular detection. Our approach distinctively leverages the DNA tetrahedron not merely as a passive scaffold, but as an

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active and organized component that pre-organizes the molecular recognition elements, thereby enhancing the efficiency of the subsequent HCR amplification step—a synergy that has been less explored in previous biosensor designs.

This study aims to develop a novel biosensing platform that leverages the synergistic combination of DNA tetrahedron nanostructures, AuNPs, and HCR amplification for specific and sensitive detection of miRNA21. The research demonstrates a comprehensive approach from fundamental design to experimental optimization, addressing technical challenges and establishing a robust methodology for biomarker detection. It addresses this by implementing a step-wise assembly strategy and rigorous buffer condition optimization. This study therefore not only presents a sensor but also provides a detailed methodological framework for constructing such complex bio-nano interfaces, which we anticipate will be informative for the broader field of DNA-based diagnostics. Consequently, this manuscript details not only the final performance metrics of the biosensor but also the critically optimized protocols for its construction, which we envision will serve as a valuable reference for the community.

2. Methods and Materials

2.1. Materials and Reagents

All DNA strands were synthesized with HPLC purification. The sequences were designed as follows:

S1:5'-HS-ATTTATCACCCGCCATAGTAGACGTATCACCAGGCAGTTGAGACGAACATTCCTAAGTCTGAA-3',

S2:5'-HS-ACATGCGAGGGTCCAATACCGACGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCG-3',

S3:5'-HS-ACTACTATGGCGGGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCC-3',

S4:5'-ACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCG-3',

mimicDNA21: TAGCTTATCAGACTGATGTTGA,

mimicDNA17: CAAAGTGCTTACAGTGCAGGTAG,

mimicDNA145: GTCCAGTTTTCCCAGGAATCCCT,

mimicDNA205: TCCTTCATTCCACCGGAGTCTG,

mimicDNA451a: AAACCGTTACCATTACTGAGTT,

Probe: GGCTAATGAAGTTCAACATCAG,

S4-apt:TCTGATAAGCTAAAAAACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCG,

linker: ACTTCATTAGCCCTGATGTTGA.

Chloroauric acid (HAuCl4), trisodium citrate, magnesium chloride hexahydrate (MgCl2·6H2O), and other chemicals were obtained from Sigma-Aldrich. All solutions were prepared using nuclease-free water.

2.2. AuNP Synthesis and Characterization

AuNPs were synthesized using the citrate reduction method [6]. Briefly, 100 mL of 0.01% HAuCl4 was brought to a boil under reflux with vigorous stirring. Then, 2 mL of 1% trisodium citrate solution was rapidly injected into the boiling solution. The reaction was allowed to proceed for 15 minutes, during which the solution color transitioned from pale yellow to deep red, indicating the formation of monodisperse spherical AuNPs. The resulting AuNP colloid was characterized by UV-Vis

spectroscopy, which showed a characteristic surface plasmon resonance (SPR) peak at approximately 520 nm. The average particle size was estimated to be around 20 nm based on the peak position and the full width at half maximum, and the concentration was estimated to be in the low nanomolar range (~2.5 nM) using established extinction coefficients, which was crucial for subsequent functionalization steps.

2.3. DNA Tetrahedron Assembly and Characterization

The four thiol-modified DNA strands were mixed in equimolar ratios (1 μM each) in TAE/Mg2+ buffer (8 mM Mg2+). The mixture underwent thermal annealing: 95 ℃ for 5 min, rapid cooling to 4 ℃ for 30 min, followed by incubation at 25 ℃ for 1 h. Successful assembly was verified through 12% native PAGE electrophoresis [7]. The clear, single band with significantly retarded mobility in the lane containing the mixture (Lane 1), compared to the fast-migrating individual strands (Lanes 2-5), confirms the formation of a monodisperse, high-molecular-weight nanostructure, indicative of a high assembly yield.

2.4. Detection Protocol and Optimization

The detection process involved the systematic optimization of key parameters—including probe concentration, reaction time, and washing stringency—to maximize the signal-to-noise ratio. Following optimization, various concentrations of mimicDNA21 were tested in triplicate to establish the calibration curve. Fluorescence detection was performed using a fluorometer with FAM excitation/emission set at 492/518 nm.

3. Results and Discussion

3.1. Detection Principle and Mechanism

The fundamental detection principle of our biosensing platform relies on a sophisticated integration of DNA nanotechnology and signal amplification strategies. As illustrated in Figure 1, the system operates through four sequential mechanisms: First, DNA tetrahedron structures are self-assembled from four specifically designed thiol-modified DNA strands, creating a stable three-dimensional framework with precise spatial organization. These tetrahedrons are then anchored to gold nanoparticles through Au-S bonds, forming a uniform sensing interface. Second, in the presence of the target molecule (mimicDNA21), specific hybridization occurs with complementary sequences on the DNA tetrahedron platform. Third, this recognition event triggers the hybridization chain reaction (HCR), where two hairpin probes undergo alternating hybridization to form extended double-stranded DNA polymers. Finally, this assembly incorporates numerous fluorescent molecules (FAM-labeled probes) that were initially quenched by the proximity to AuNPs, resulting in dramatic fluorescence signal amplification. The intensity of the recovered fluorescence is directly proportional to the concentration of the target miRNA21, enabling quantitative detection. This design strategically decouples the target recognition event from the signal amplification module. The DNA tetrahedron ensures that the initiator strands for HCR are presented in a spatially controlled and accessible manner, while the HCR process transduces a single binding event into the assembly of a long DNA polymer, carrying numerous fluorophores. The initial fluorescence quenching by the AuNPs via a Förster resonance energy transfer (FRET) mechanism ensures a low background, and the subsequent displacement and polymerization during HCR physically separate the fluorophores from the AuNP surface, leading to a dramatic ‘turn-on’ fluorescent signal.

This multi-component architecture offers several distinct advantages over simpler probe designs. Firstly, the separation of the recognition element (on the tetrahedron) from the amplification machinery (HCR hairpins in solution) allows for independent optimization of each module. Secondly, the tetrahedron’s scaffold ensures that every captured target molecule is primed to initiate an HCR event, maximizing the efficiency of signal conversion. Lastly, the use of AuNPs as a universal quenching platform simplifies the design and lowers cost compared to molecular quencher-based systems.

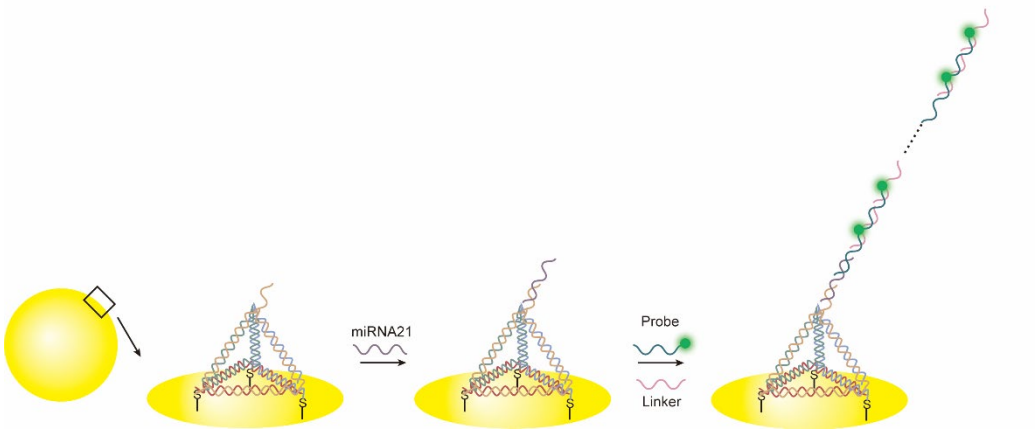

Figure 1 Schematic illustration of the DNA tetrahedron-based biosensing platform showing the integration of DNA nanotechnology, gold nanoparticles, and HCR amplification for miRNA21 detection.

3.2. DNA Tetrahedron Assembly and Validation

Successful assembly of DNA tetrahedrons was confirmed through polyacrylamide gel electrophoresis. As shown in Figure 2, native PAGE analysis clearly demonstrated the assembly of the DNA tetrahedron structure. The tetrahedron structure showed significantly retarded mobility compared to individual DNA strands, consistent with its larger hydrodynamic size and rigid three-dimensional structure. This validation was crucial for ensuring the proper formation of the sensing platform.

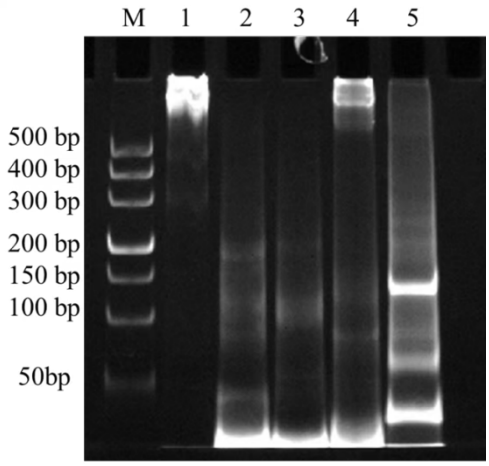

Figure 2 Native PAGE analysis of DNA tetrahedron assembly. Lane 1: S1+S2+S3+S4; Lane 2: S1; Lane 3: S2, Lane 4: S3, Lane 5: S4; Tetrahedron: Assembled structure showing retarded mobility.

3.3. Performance Optimization and Sensitivity Assessment

The analytical performance of the biosensor was rigorously evaluated under conditions optimized through iterative investigation of key parameters, including DNA probe concentration, Mg^{2+} concentration, and HCR reaction kinetics. The optimal configuration was determined to be: a probe concentration of 12 μL of 10 μM solution, an Mg^{2+} concentration of 8 mM to ensure tetrahedron stability, and an HCR reaction time of 2 hours at 37 °C to ensure complete amplification while minimizing non-specific background.

Under these optimized conditions, the biosensor exhibited exceptional performance for the detection of mimicDNA21. As illustrated in Figure 3, the platform achieved a wide linear dynamic range from 100 fM to 100 pM, spanning five orders of magnitude, with an excellent coefficient of determination ($R^2 = 0.9934$). The detection limit was calculated to be as low as 1 fM, underscoring the exceptional signal amplification efficiency of the integrated HCR process.

This level of sensitivity represents a significant advancement. When contextualized within the

landscape of recent miRNA sensing technologies, our platform's performance is highly competitive. It surpasses the sensitivity of many AuNP-based colorimetric sensors by several orders of magnitude and rivals that of certain qPCR-based assays and other sophisticated methods [8], all while operating under isothermal conditions and foregoing the need for expensive enzymes. The key advantage of our approach lies in this isothermal and enzyme-free amplification mechanism, which significantly simplifies the operational procedure and reduces cost. We postulate that the remarkable sensitivity and wide linear range are direct benefits of the sophisticated platform design. The pre-organization of the HCR initiator strands on the rigid DNA tetrahedron scaffold likely creates a locally high effective concentration, facilitating the kinetics of the HCR initiation step. Furthermore, the precise spatial control over probe density afforded by the tetrahedron prevents surface crowding and ensures efficient hybridization and polymerization, allowing the signal amplification to remain efficient across a wide range of target concentrations without early saturation. The high R^2 value indicates a reliable and well-behaved dose-response relationship, which is paramount for the accurate quantification of target biomarkers in practical applications.

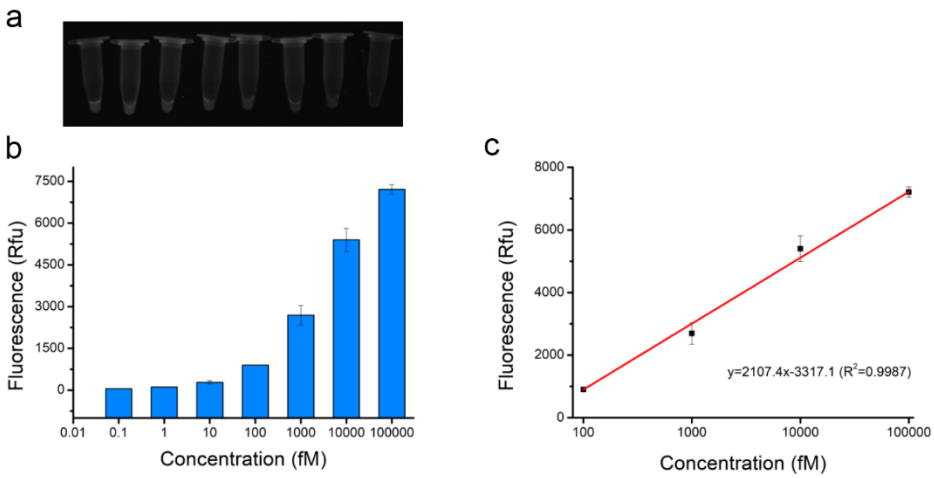

Figure 3 (a) shows the fluorescence signals observed using a gel imaging system, with concentrations decreasing from 100,000 fM to 0 from right to left. (b) displays the corresponding fluorescence intensity histogram detected by a real-time PCR instrument. (c) illustrates the linear relationship between concentrations ranging from 100 fM to 100,000 fM.

3.4. Specificity Evaluation

The biosensor exhibited outstanding specificity for mimicDNA21, with minimal cross-reactivity against non-target sequences. To rigorously evaluate specificity, we introduced 1 pM of various non-target miRNA mimics (including mimicDNA17, mimicDNA145, mimicDNA205, and mimicDNA451a, corresponding to miR-17, miR-145, miR-205, and miR-451a, respectively) into the system, as shown in Figure 4. Fluorescence measurements revealed negligible signals for all non-target groups compared to the mimicDNA21 experimental group. The strongest signal among non-target sequences---observed for mimicDNA17 - reached only 4.12% of that for mimicDNA21, demonstrating highly selective target recognition [9]. These results underscore the platform's ability to distinguish miRNA21 from structurally similar miRNAs, a critical attribute for clinical applications involving complex biological samples.

The exceptional specificity, as evidenced by the minimal signals from non-target miRNAs, can be attributed to the precise base pairing required not only for the initial target capture on the tetrahedron but also for the meta-stable hairpin probes of the HCR to unfold and propagate. The spatial confinement of the capture probes on the rigid tetrahedron framework may further reduce non-specific interactions and improve discrimination against single-base mismatches. While the structural basis for the minor signal from mimicDNA17 (4.12%) warrants further investigation, it underscores the importance of probe sequence design. Crucially, this low level of cross-reactivity confirms that our platform can effectively tolerate the presence of high concentrations of non-target miRNAs, a

critical prerequisite for analyzing real biological samples where hundreds of different miRNA species coexist. The current level of specificity is already highly promising for clinical applications. Such performance aligns with the requirements for clinical diagnostics, where distinguishing closely related biomarkers is essential [10].

Furthermore, the rigid three-dimensional structure of the DNA tetrahedron is known to confer enhanced nuclease resistance and structural stability compared to linear single-stranded DNA probes. This inherent stability of the core sensing element suggests a favorable foundation for developing robust biosensors, though long-term storage stability and performance in complex matrices warrant future investigation.

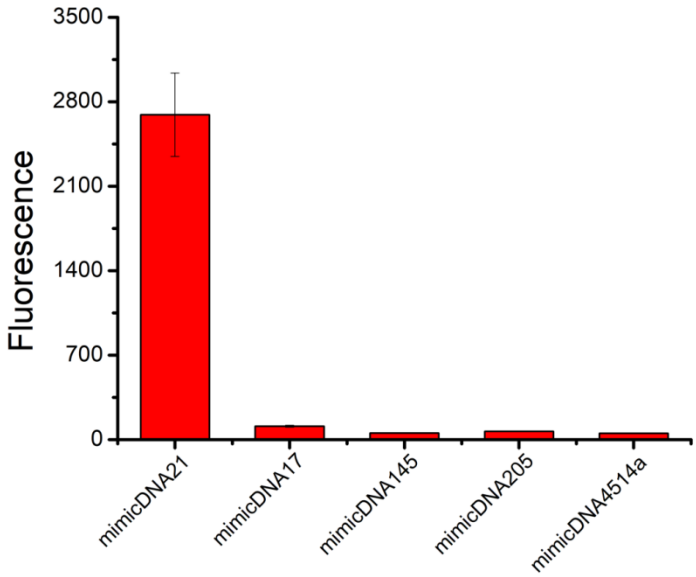

Figure 4 Specificity evaluation of the DNA tetrahedron-based biosensing platform for miRNA21 detection. The histogram illustrates the fluorescence signals obtained in response to 1 pM mimicDNA21 compared to various non-target miRNA mimics (mimicDNA17, mimicDNA145, mimicDNA205, and mimicDNA451a).

4. Conclusion

This study has successfully established and characterized a novel biosensing platform that synergistically integrates the spatial precision of DNA tetrahedrons, the excellent quenching properties of AuNPs, and the powerful, enzyme-free amplification of HCR for the ultrasensitive detection of miRNA21. The 'turn-on' fluorescence signal, triggered by target-specific HCR polymerization that displaces fluorophores from the AuNP surface, provides a robust and quantitative readout. The integration of DNA nanotechnology with HCR amplification strategy resulted in a detection system with exceptional sensitivity (1 fM detection limit) and a wide linear range (100 fM - 100 pM). The rigid three-dimensional structure of DNA tetrahedrons provided enhanced stability and precise spatial organization, while AuNP conjugation facilitated efficient signal modulation. The biosensor presents significant potential for clinical applications in cancer diagnostics and monitoring. Beyond the detection of miRNA21, the modularity of this platform underscores its utility as a versatile biosensing schema. The DNA tetrahedron can be reconfigured with different probe sequences, and the HCR system can be adapted accordingly, making it a promising tool for a wide array of molecular diagnostics. Future work will be directed along three primary avenues: first, a comprehensive evaluation of biosensor's shelf-life stability and reproducibility across different production batches to assess its commercial viability, second, adapting the platform for direct miRNA detection in complex biological samples such as serum and cell lysates, which will require further engineering to mitigate matrix effects and nuclease degradation [11]; and third, developing a multiplexed detection array capable of simultaneous profiling of a panel of cancer-related miRNAs (e.g., miR-21, miR-155, miR-210) on a single chip by spatially patterning different tetrahedron probes, a feat achievable through the modular design of our platform [12]. The journey from a robust

laboratory prototype to a validated clinical tool presents distinct challenges, but the foundational work presented herein provides a strong and promising starting point.

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