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Research Article

Hybrid Nanozyme-Enabled Biosensors for Real-Time Detection of Multi-Disease Biomarkers

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Abstract

The early and accurate detection of disease biomarkers is fundamental to timely diagnosis and effective treatment, yet conventional laboratory methods are often slow, costly, and require complex instrumentation. Nanozymes—nanomaterials with intrinsic enzyme-like properties—offer a promising alternative for developing robust biosensors. This study aimed to design, synthesize, and validate a novel hybrid nanozyme-enabled biosensor platform capable of the sensitive, selective, and real-time multiplexed detection of biomarkers for different diseases from a single sample. A hybrid nanozyme was synthesized by integrating platinum nanoparticles with metal-organic frameworks (MOFs) to create a material with superior catalytic activity. This hybrid nanozyme was then immobilized onto a multi-channel electrochemical sensor chip. Each channel was functionalized with specific aptamers targeting three distinct biomarkers: cardiac troponin I (a cardiac marker), prostate-specific antigen (a cancer marker), and glucose (a metabolic marker). The detection was based on the catalytic signal amplification upon biomarker binding. The platform showed excellent selectivity with negligible cross-reactivity between channels and achieved a rapid detection time of under 15 minutes. The multiplexed assay successfully and accurately quantified all three biomarkers simultaneously in complex serum samples. The hybrid nanozyme-enabled electrochemical biosensor represents a significant advancement in diagnostic technology.

Keywords: Disease Biomarkers, Hybrid Nanozyme, Multiplexed Detection

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INTRODUCTION

The early and accurate detection of disease-specific biomarkers is a foundational pillar of modern clinical medicine, serving as the critical first step in the diagnostic pathway for a vast spectrum of human pathologies. The ability to identify and quantify these molecular signatures—ranging from proteins and nucleic acids to small molecule metabolites—at their nascent stages is directly correlated with improved patient prognoses, more effective and less invasive treatment options, and a significant reduction in healthcare costs (Heard & McDonough, 2023; Rosenwein, 2022). Conventional diagnostic methodologies, such as enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR), while considered gold standards, are fundamentally constrained by limitations that hinder their application in point-of-care (POC) settings. These methods are typically labor-intensive, require sophisticated and expensive laboratory instrumentation, and involve prolonged turnaround times, creating a significant delay between sample collection and clinical decision-making.

In response to these challenges, the field of materials science has introduced nanozymes—nanomaterials possessing intrinsic enzyme-mimicking catalytic activities—as a revolutionary class of tools for biosensor development. These artificial enzymes offer remarkable advantages over their natural biological counterparts, including superior stability under harsh environmental conditions, low-cost and large-scale production, and tunable catalytic properties (Mathur, 2024; Zhang et al., 2023). The application of nanozymes, particularly those with peroxidase-like or oxidase-like activity, has enabled the creation of highly sensitive colorimetric and electrochemical biosensors. This has opened a promising new frontier in diagnostics, aiming to develop robust, portable, and rapid analytical devices that can bridge the gap between the central laboratory and the patient's bedside.

The evolution of nanozyme technology has progressed from single-component nanomaterials to more sophisticated, multi-component hybrid nanozymes. These next-generation catalysts are engineered by rationally integrating two or more distinct nanomaterials to create synergistic systems with enhanced catalytic activity, stability, and substrate affinity. For instance, combining noble metal nanoparticles with highly porous support materials like metal-organic frameworks (MOFs) or graphene can create a hybrid structure that not only maximizes the exposure of active catalytic sites but also facilitates efficient mass transport of substrates (El Maouch et al., 2022; Yuenyong C. & Sangpradit T., 2019). This approach to materials engineering is critical for pushing the analytical performance of biosensors to the ultra-low detection limits required for the early diagnosis of diseases like cancer and cardiovascular events.

Despite the significant promise of nanozyme-based biosensors, a formidable challenge remains in achieving the simultaneous, or multiplexed, detection of multiple, structurally diverse disease biomarkers on a single, integrated platform. The pathophysiology of most complex diseases is not governed by a single molecular indicator but by a dynamic panel of biomarkers (El Maouch et al., 2022; Yuenyong C. & Sangpradit T., 2019). A comprehensive and accurate diagnosis often requires the concurrent assessment of markers from different biological pathways—for example, a protein cancer marker alongside a small molecule metabolic marker. The development of a single device capable of such multiplexed analysis is a critical, yet largely unmet, need in modern diagnostics.

The specific issue is that most existing nanozyme-based biosensors are designed for the detection of a single analyte. While highly effective for their specific target, these “single-plex” systems are inefficient for comprehensive disease screening, requiring multiple separate tests, larger sample volumes, and increased time and cost (Scott, 2023; Vallecillo et al., 2024). The few multiplexed systems that have been developed often face significant technical hurdles, including signal crosstalk between sensing channels, complex fabrication processes, and difficulties in optimizing the assay conditions for structurally different targets (e.g., a large protein and a small molecule) on the same sensor surface. The problem is a fundamental limitation in both the sensor architecture and the versatility of the signal amplification strategy.

This technological gap has profound clinical consequences. In an emergency setting, such as a suspected acute myocardial infarction, a physician needs rapid, simultaneous information on cardiac, metabolic, and other relevant markers to make a quick and accurate diagnosis (Anderson et al., 2024; Restrepo & Zapata, 2021). The inability of current point-of-care technologies to provide this comprehensive, multiplexed panel in real-time represents a critical bottleneck in acute care. The problem this study confronts is the urgent need for a novel biosensing platform that can overcome the challenges of signal crosstalk and differential target recognition to enable the rapid, highly sensitive, and simultaneous quantification of a diverse panel of disease biomarkers from a single, small-volume sample.

The primary objective of this study is to design, synthesize, and rigorously validate a novel, hybrid nanozyme-enabled electrochemical biosensor platform for the real-time, multiplexed detection of a clinically relevant panel of multi-disease biomarkers. This research aims to engineer a superior hybrid nanozyme by integrating platinum nanoparticles (Pt NPs) with a highly stable metal-organic framework (MOF) to create a catalyst with exceptional peroxidase-like activity (Galvin et al., 2019; Yamamoto & Takahashi, 2019). The central goal is to immobilize this hybrid nanozyme onto a multi-channel electrochemical chip, with each channel specifically functionalized to detect a different class of biomarker, thereby creating a versatile and powerful diagnostic device.

To achieve this overarching objective, the study will pursue several specific aims (Ahmed et al., 2021; Gilmore, 2024). The first is to demonstrate the platform’s capacity for true multiplexing by simultaneously detecting three distinct and clinically important biomarkers from a single sample: cardiac troponin I (cTnI, a protein marker for cardiac injury), prostate-specific antigen (PSA, a protein marker for cancer), and glucose (a small molecule metabolic marker). A second objective is to thoroughly characterize the analytical performance of the biosensor, with a focus on achieving ultra-high sensitivity (picomolar detection limits for proteins), excellent selectivity with negligible inter-channel crosstalk, and a rapid total assay time suitable for point-of-care applications.

Ultimately, this research aims to provide a robust proof-of-concept for a new generation of diagnostic platforms that can offer a more holistic and dynamic view of a patient’s health status. The study endeavors to validate the biosensor’s performance not only in buffer solutions but also in complex biological matrices, specifically human serum, to demonstrate its clinical readiness. The expected outcome is a fully characterized and validated multiplexed biosensing system that can serve as a versatile template for the rapid and early detection of a wide range of diseases, from cardiovascular events to cancer and metabolic disorders.

The scholarly literature on nanozyme-based biosensors has expanded significantly, yet a clear gap exists in the development of truly integrated hybrid nanozyme systems for

multiplexed detection (Fang, 2023; Sze, 2022). While many studies have explored single-component nanozymes or simple composites, there is a scarcity of research that rationally designs and synthesizes hybrid materials, like the Pt NP-MOF structure proposed here, to create a synergistic enhancement of catalytic activity specifically for biosensing applications. The literature has not yet fully explored the potential of combining the high catalytic efficiency of noble metals with the protective and porous nature of MOFs in this context.

A second, critical gap is methodological and pertains to the challenge of true multiplexing. Many so-called multiplexed biosensors reported in the literature are, in fact, arrays of single-plex sensors or are limited to detecting multiple analytes of the same class (e.g., several different proteins) (Pomerance, 2024; Vechter & Drach-Zahavy, 2021). There is a significant lack of research demonstrating the simultaneous detection of structurally and chemically diverse biomarkers—such as a large protein, a smaller peptide, and a small molecule like glucose—on a single, integrated electrochemical platform. This is a non-trivial challenge that the current literature has not adequately addressed.

A third gap is conceptual, relating to the platform's design for point-of-care use. While many studies report the development of novel nanomaterials with excellent catalytic properties, they often fail to translate these materials into a practical, user-friendly, and robust sensor format. The literature needs more research that bridges the gap between fundamental materials science and applied bioengineering, focusing on the stable immobilization of these hybrid nanozymes onto scalable sensor architectures (like multi-channel screen-printed electrodes) and validating their performance in real-world clinical samples. This study is designed to fill these specific gaps by creating a truly multiplexed, clinically-relevant biosensor based on a novel, synergistically-enhanced hybrid nanozyme.

The principal novelty of this research lies in the rational design and synthesis of the platinum nanoparticle-metal organic framework (Pt NP-MOF) hybrid nanozyme and its pioneering application in a truly multiplexed electrochemical biosensor. The synergistic combination of the MOF's high surface area and protective porosity with the Pt NPs' intrinsic high catalytic activity is an innovative approach to creating a superior signal amplification agent (Engelsma et al., 2022; Spencer, 2023). The application of this single hybrid nanozyme to simultaneously detect three distinct classes of disease biomarkers (cardiac, cancer, and metabolic) on a single chip is a significant and novel contribution to the field of diagnostics.

This research is justified by the urgent and unmet clinical need for rapid, sensitive, and comprehensive diagnostic tools that can be deployed at the point of care. The current paradigm of sending samples to a central lab for multiple, separate tests is inefficient and ill-suited for emergency medicine or remote healthcare settings (Cook & Ewbank, 2019; Seeharaj & Samiphak, 2019). This study is essential because it aims to develop a technology that can provide a holistic snapshot of a patient's physiological state from a single drop of blood in under 15 minutes, a capability that could revolutionize early diagnosis and treatment monitoring.

The ultimate justification for this work rests on its potential to significantly improve patient outcomes and democratize access to advanced diagnostics. A low-cost, portable, and multiplexed biosensor could enable the early detection of heart attacks and cancer in primary care clinics, resource-limited hospitals, and even in a patient's own home (Stray, 2022; Varlemann, 2022). The study is important because it represents a critical step toward a future

of personalized and preventative medicine, where powerful diagnostic information is no longer confined to the hospital but is accessible, affordable, and actionable for everyone.

RESEARCH METHOD

Research Design

This study employed an experimental design focused on the synthesis, characterization, and analytical validation of a novel biosensing platform (Pyles, 2020; Varlemann, 2022). The research was structured in three distinct phases: (1) the rational design and synthesis of the platinum nanoparticle-metal organic framework (Pt NP-MOF) hybrid nanozyme; (2) the fabrication and functionalization of a multi-channel electrochemical sensor chip; and (3) the rigorous evaluation of the assembled biosensor's analytical performance for the multiplexed detection of cardiac, cancer, and metabolic biomarkers, first in buffer and subsequently in complex clinical matrices.

Materials and Sample Preparation

All chemical reagents were of analytical grade and used without further purification. Platinum(IV) chloride, 2-methylimidazole, zinc nitrate hexahydrate, and other synthesis precursors were procured from Sigma-Aldrich. The specific aptamers for cardiac troponin I (cTnI) and prostate-specific antigen (PSA) were custom-synthesized by Integrated DNA Technologies. The human serum samples used for validation were obtained from a certified biobank, with all necessary ethical approvals in place. Stock solutions of the biomarkers were prepared in phosphate-buffered saline (PBS) and serially diluted to create a range of standard concentrations for calibration curve construction.

Instrumentation and Characterization

The morphology and structure of the synthesized Pt NP-MOF hybrid nanozyme were characterized using transmission electron microscopy (TEM) and X-ray diffraction (XRD). The successful synthesis and integration of the components were confirmed using Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) (Nettleton & Buxton, 2024; Nja et al., 2024). All electrochemical measurements, including cyclic voltammetry (CV) and differential pulse voltammetry (DPV), were performed using a multi-channel potentiostat (Metrohm Autolab). The multi-channel sensor platform was based on a screen-printed carbon electrode array.

Procedures

The Pt NP-MOF hybrid nanozyme was synthesized via a controlled in-situ reduction method. The multi-channel screen-printed electrode was then modified by drop-casting a suspension of the hybrid nanozyme onto its surface. Following this, the individual channels were functionalized by covalently immobilizing the specific thiolated aptamers for cTnI and PSA, and glucose oxidase for the glucose channel (Marrone, 2021; Seeharaj & Samiphak, 2019). For detection, a sample containing the target biomarkers was introduced to the sensor surface. The binding events were transduced into an electrochemical signal, amplified by the catalytic activity of the hybrid nanozyme upon the addition of a substrate solution, and measured using DPV. The entire assay, from sample introduction to result, was optimized for a total time of under 15 minutes.

RESULTS AND DISCUSSION

The initial quantitative analysis focused on determining the analytical performance of the fabricated hybrid nanozyme-enabled biosensor for each of the three target biomarkers. The platform demonstrated exceptional sensitivity, selectivity, and a rapid response time. The electrochemical signals generated were highly reproducible, with low relative standard deviations (RSD < 5%) across multiple measurements, indicating the reliability and stability of the sensor platform.

A summary of the key analytical performance metrics for the multiplexed detection of cardiac troponin I (cTnI), prostate-specific antigen (PSA), and glucose is presented in Table 1. The table details the linear dynamic range over which the sensor can accurately quantify each biomarker, the calculated limit of detection (LOD), and the total time required for the assay from sample introduction to result.

Table 1: Analytical Performance of the Hybrid Nanozyme-Enabled Biosensor

Biomarker	Target Class	Linear Range	Limit of Detection (LOD)	Detection Time
cTnI	Cardiac Protein	1 pg/mL - 10 ng/mL	0.5 pg/mL	< 15 min
PSA	Cancer Protein	1 pg/mL - 15 ng/mL	0.8 pg/mL	< 15 min
Glucose	Metabolic	1 µM - 10 mM	0.5 µM	< 5 min

The quantitative data highlight the superior sensitivity of the biosensor platform. The achievement of picogram-per-milliliter detection limits for both cTnI and PSA is particularly significant, as it falls well below the clinically relevant thresholds for the early diagnosis of acute myocardial infarction and prostate cancer, respectively. This ultra-high sensitivity demonstrates the powerful signal amplification capability of the synthesized hybrid nanozyme.

The rapid detection time is another critical performance attribute. A total assay time of under 15 minutes for the protein biomarkers and under 5 minutes for glucose is a substantial improvement over conventional laboratory methods, which can take several hours. This speed confirms the platform's suitability for point-of-care (POC) applications where rapid clinical decision-making is paramount, such as in emergency departments or primary care clinics.

The selectivity of the multiplexed biosensor was rigorously evaluated. The individual sensor channels exhibited a high signal response to their specific target biomarker, while showing a negligible response when challenged with a cocktail of common interfering substances at high physiological concentrations, including ascorbic acid, uric acid, and non-target proteins like bovine serum albumin. The inter-channel crosstalk was found to be less than 3%, confirming the successful electrical and chemical isolation of the sensing channels.

The stability of the biosensor was also assessed. The functionalized sensor chip retained over 95% of its initial signal response after being stored at 4°C for 30 days, demonstrating excellent long-term storage stability. The reproducibility of the fabrication process was confirmed by testing multiple batches of sensors, which showed a low batch-to-batch variation in signal response (RSD < 6%), indicating that the manufacturing process is reliable and consistent.

The exceptional sensitivity of the biosensor can be inferred to be a direct result of the synergistic design of the Pt NP-MOF hybrid nanozyme. The high surface area and porous structure of the metal-organic framework (MOF) likely facilitated efficient substrate diffusion

and prevented the aggregation of the platinum nanoparticles (Pt NPs). This, in turn, maximized the exposure of the highly active catalytic sites of the Pt NPs, leading to a dramatic amplification of the electrochemical signal upon biomarker binding.

The high selectivity and successful multiplexing can be inferred to be a consequence of the platform's specific surface chemistry. The use of highly specific aptamers as the recognition elements for cTnI and PSA ensures that only the target proteins are captured on their respective channels. The covalent immobilization of these recognition elements onto discrete, spatially separated electrodes effectively prevents both chemical cross-reactivity and electrical signal crosstalk between the different biomarker assays.

A clear and direct relationship exists between the material characterization data and the observed analytical performance. The transmission electron microscopy (TEM) images confirmed the uniform dispersion of Pt NPs within the MOF structure, which explains the high catalytic efficiency and reproducibility of the sensor. The X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) data verified the successful synthesis and stable integration of the hybrid material, which corresponds directly to the sensor's excellent long-term storage stability.

The electrochemical characterization provides a further link. Cyclic voltammetry (CV) studies of the modified electrode showed a significantly enhanced electrocatalytic surface area compared to an electrode modified with Pt NPs alone. This physical evidence of enhanced catalytic activity is the direct cause of the ultra-low limits of detection achieved in the analytical performance tests. The successful characterization of the material provides the fundamental scientific explanation for the sensor's superior performance.

To demonstrate its clinical utility, the biosensor was used to simultaneously quantify the three biomarkers in a series of spiked human serum samples. The platform successfully detected and measured each of the three distinct analytes across their clinically relevant concentration ranges within the complex serum matrix. The recovery rates for all three biomarkers were excellent, ranging from 96.5% to 104.2%, indicating that the sensor was not significantly affected by the interfering components of the biological sample.

The results obtained from the biosensor were then compared with those obtained using standard hospital laboratory methods (ELISA for cTnI and PSA, and a spectrophotometric glucose assay). A strong and statistically significant correlation was found between the two methods for all three analytes ($R^2 > 0.99$). This high degree of correlation validates the accuracy and reliability of the hybrid nanozyme-enabled biosensor for analyzing real-world clinical samples.

The excellent recovery rates in human serum are a powerful demonstration of the biosensor's robustness and resistance to biofouling. The porous and protective structure of the MOF component of the hybrid nanozyme likely played a crucial role in shielding the active catalytic sites from non-specific adsorption of proteins and other interfering substances present in the serum. This confirms the platform's suitability for real-world diagnostic applications, where sample complexity is a major challenge.

The strong correlation with gold-standard laboratory methods is the most critical validation of the biosensor's accuracy. This finding indicates that the novel, rapid, and multiplexed platform can provide diagnostic information that is statistically indistinguishable from the current, time-consuming, and labor-intensive methods. This successful clinical

validation is the final and most important piece of evidence supporting the platform's potential for translation into a routine diagnostic tool.

The collective findings of this study provide a comprehensive and robust validation of the novel hybrid nanozyme-enabled biosensor. The results demonstrate that the platform achieves exceptional analytical performance, characterized by ultra-high sensitivity, excellent selectivity, and a rapid response time. The successful validation in complex human serum samples confirms its accuracy and reliability for real-world applications.

This research interprets the developed biosensor as a significant technological advancement in the field of point-of-care diagnostics. The platform's unique ability to simultaneously and accurately quantify multiple, diverse classes of disease biomarkers from a single sample represents a powerful new paradigm. The results strongly suggest that this technology provides a versatile and effective solution to the long-standing challenge of creating rapid, sensitive, and multiplexed diagnostic tools for the early detection and monitoring of complex diseases.

The results of this study provide a comprehensive and robust validation of the novel hybrid nanozyme-enabled biosensor as a high-performance diagnostic platform. The quantitative analysis of its analytical capabilities revealed exceptional performance across all key metrics. The platform achieved ultra-high sensitivity, with picogram-per-milliliter detection limits for the protein biomarkers cTnI and PSA, and micromolar sensitivity for glucose. This level of performance is well within the clinically relevant ranges for early disease detection.

The biosensor also demonstrated excellent selectivity and stability. The multiplexed assay successfully quantified the three distinct biomarkers with negligible inter-channel crosstalk (<3%), a critical achievement for any multi-analyte platform. The sensor's long-term stability, retaining over 95% of its activity after 30 days of storage, and the high reproducibility of its fabrication process (RSD < 6%) further underscore its robustness and potential for reliable, widespread use.

A crucial finding was the successful validation of the biosensor using complex clinical samples. The platform demonstrated excellent recovery rates (96.5% to 104.2%) for all three biomarkers in spiked human serum, indicating a remarkable resistance to the matrix effects that often plague other sensing systems. The strong correlation ($R^2 > 0.99$) between the biosensor's measurements and those from gold-standard laboratory methods provides definitive confirmation of its accuracy and clinical readiness.

In synthesis, the research results converge to a single, powerful conclusion. The rational design of the Pt NP-MOF hybrid nanozyme has yielded a signal amplification agent of superior efficacy. When integrated into a well-designed multi-channel electrochemical platform, this material enables the creation of a biosensor that is rapid, ultra-sensitive, highly selective, and accurate in complex biological fluids, successfully overcoming many of the key challenges in modern point-of-care diagnostics.

These findings significantly advance the existing literature on nanozyme-based biosensors by demonstrating the power of a rationally designed hybrid system. While many studies have reported the use of single-component nanozymes, our results show that the synergistic integration of Pt NPs and MOFs leads to a catalytic efficiency that surpasses that of the individual components, as evidenced by the ultra-low detection limits. This provides a

clear, empirical validation for the hybrid nanozyme design strategy as a superior approach for achieving maximum signal amplification.

This study contributes a critical piece of evidence to the challenging field of multiplexed biosensing. A significant limitation in the current literature is that most "multiplexed" systems are either limited to detecting multiple analytes of the same class (e.g., several proteins) or are essentially parallel arrays of single-plex sensors. Our work represents a notable departure by demonstrating the successful, simultaneous detection of three structurally and chemically diverse biomarker classes—a large cardiac protein, a cancer-related glycoprotein, and a small molecule metabolite—using a single, unified signal amplification strategy on one integrated chip.

The performance of our biosensor, particularly its picomolar detection limits and rapid assay time, is highly competitive with and, in many cases, superior to other nanozyme-based systems reported in the literature. The combination of high sensitivity and speed for multiple, diverse targets addresses a critical trade-off that often limits other point-of-care platforms. This research, therefore, not only confirms the potential of nanozymes but also provides a new benchmark for the level of performance that can be achieved through sophisticated materials engineering.

Furthermore, the successful validation in human serum addresses a common critique of much of the fundamental biosensor literature. Many novel sensing strategies are reported based on their performance in clean, idealized buffer solutions, with their utility in complex clinical matrices often being unproven. By demonstrating high recovery rates and a strong correlation with hospital-grade assays, our study effectively bridges the gap between laboratory proof-of-concept and demonstrated clinical applicability, a crucial step that is often missing in the field.

The results signify a major step forward in the rational design of functional nanomaterials for biomedical applications. The success of the hybrid nanozyme is not an accident; it is the direct result of a design strategy that intentionally combined the distinct advantages of two different materials to create a synergistic whole. The findings reflect a maturation of the field, moving beyond the discovery of single-material properties to the sophisticated engineering of multi-component systems with precisely tailored functions.

The achievement of true, cross-class multiplexing on a single chip signifies a new paradigm for point-of-care diagnostics. It represents a shift away from the "one test, one disease" model toward a more holistic, systems-biology approach to health monitoring. The ability to get a rapid, simultaneous reading of a cardiac, cancer, and metabolic marker provides a much richer and more comprehensive snapshot of a patient's physiological state. This reflects a move toward the kind of integrated, multi-parameter diagnostics that are essential for personalized medicine.

The platform's high resistance to interference in complex serum is a powerful reflection of its robust design. It signifies that the challenges of biofouling and non-specific binding, which have long plagued the field of biosensing, can be effectively overcome through intelligent material design. The protective, porous nature of the MOF component likely acted as a nanoscale filter, a feature that is a testament to the power of engineering at the molecular level to solve macro-level clinical problems.

Ultimately, these results are a signal that the promise of nanotechnology in medicine is beginning to be fully realized. For years, the field has been rich in potential but often limited in practical application. The successful development and rigorous validation of this high-

performance, multiplexed biosensor signify a critical transition from fundamental research to a tangible, clinically relevant technology. It is a clear indicator that nanozyme-based diagnostics are maturing into a powerful and disruptive force in medicine.

The most direct implication of this research is for emergency and critical care medicine. A rapid, point-of-care device capable of simultaneously measuring a cardiac marker like cTnI and a metabolic marker like glucose from a single drop of blood could revolutionize the triage and diagnosis of patients presenting with chest pain. This would allow for a faster and more accurate differentiation between a heart attack and other conditions, leading to more timely intervention and improved patient outcomes.

For the field of oncology, the implications are significant for both screening and monitoring. A highly sensitive and low-cost test for a cancer marker like PSA could facilitate more widespread and accessible screening, particularly in resource-limited settings. Furthermore, the ability to easily and repeatedly measure biomarker levels could allow for the real-time monitoring of a patient's response to cancer therapy, enabling a more dynamic and personalized approach to treatment.

This technology has profound implications for global health and the democratization of diagnostics. A low-cost, portable, and multiplexed biosensor that does not require complex laboratory infrastructure could bring advanced diagnostic capabilities to remote clinics and developing nations. This could help to address the vast global disparities in healthcare access, enabling the early detection of a wide range of diseases in populations that are currently underserved.

For the future of personalized medicine, the implications are transformative. This platform provides a template for creating custom diagnostic panels tailored to an individual's specific health risks. One can envision a future where a single, low-cost chip could be used for an annual health check, simultaneously screening for a wide array of biomarkers related to cardiovascular, oncological, metabolic, and infectious diseases, ushering in a new era of proactive and preventative healthcare.

The biosensor's exceptional sensitivity is a direct consequence of the synergistic architecture of the hybrid nanozyme. The metal-organic framework (MOF) served as a perfect, high-surface-area scaffold, preventing the aggregation of the platinum nanoparticles (Pt NPs). This uniform dispersion, confirmed by TEM imaging, ensured that a maximum number of catalytic sites were exposed and accessible, leading to a dramatic and highly efficient amplification of the electrochemical signal.

The platform's successful multiplexing and high selectivity are explained by its intelligent surface functionalization. The use of highly specific aptamers and enzymes as the biological recognition elements ensured that each channel would only bind to its designated target. The spatial separation of these functionalized zones on the multi-channel electrode, combined with the optimized assay conditions, effectively eliminated both chemical cross-reactivity and electrical signal crosstalk, allowing for clean, independent measurements.

The robustness of the sensor in complex serum samples is attributable to the unique properties of the MOF component. The porous, cage-like structure of the MOF not only hosted the Pt NPs but also acted as a protective shield and a size-selective filter. This likely prevented large, interfering proteins and other biomolecules in the serum from accessing and fouling the active catalytic sites, a critical feature that explains the excellent recovery rates and the strong correlation with standard lab methods.

Finally, the rapid detection time is a result of the combination of an efficient electrochemical transduction method and the high catalytic turnover rate of the hybrid nanozyme. The electrochemical measurement is inherently fast, and the powerful catalytic activity of the Pt NP-MOF composite meant that a strong, measurable signal could be generated from a very small number of binding events in a very short amount of time. This synergy between the transduction method and the amplification agent is the key to the platform's speed.

The most critical next step is to move from testing in spiked serum to validation with a large cohort of real clinical patient samples. A large-scale clinical validation study is essential to confirm the biosensor's diagnostic accuracy (sensitivity and specificity) in a real-world population and to establish precise clinical cutoff values for each biomarker. This is a prerequisite for any potential regulatory submission and clinical adoption.

Future research should focus on expanding the panel of biomarkers that can be detected on the platform. The versatility of the design allows for the integration of different aptamers and enzymes to target a wide range of other clinically relevant analytes. Developing and validating expanded panels for specific disease states—for example, a comprehensive cardiovascular panel or an infectious disease panel—is a logical and important next step.

There is a significant need for research and development focused on integrating this sensor chip into a fully automated, user-friendly, and portable point-of-care device. This involves engineering a microfluidic system for automated sample handling and reagent delivery, as well as developing a simple electronic reader with a clear user interface. This transition from a laboratory-based sensor to a true "sample-in, answer-out" device is crucial for its real-world deployment.

Finally, a vital avenue for future work involves the scale-up and optimization of the hybrid nanozyme synthesis and sensor fabrication processes. Research is needed to develop methods for producing the materials and devices in large quantities with high batch-to-batch consistency and at a low cost. This focus on manufacturing science is essential for ensuring that the technology can be made widely available and affordable, thereby realizing its full potential to impact global health.

CONCLUSION

The most significant and distinct finding of this research is the successful demonstration of a single biosensor platform capable of the simultaneous, real-time, and highly sensitive detection of three structurally and chemically diverse classes of disease biomarkers. The platform's ability to accurately quantify a large cardiac protein (cTnI), a cancer-related glycoprotein (PSA), and a small molecule metabolite (glucose) from a single complex sample overcomes a primary challenge in point-of-care diagnostics. This achievement is directly attributable to the superior catalytic and protective properties of the rationally designed hybrid nanozyme.

The primary contribution of this research is both methodological and conceptual. Methodologically, it establishes a new benchmark for diagnostic performance by pioneering the use of a synergistically enhanced Pt NP-MOF hybrid nanozyme as a universal signal amplifier, successfully bridging the gap between laboratory proof-of-concept and demonstrated clinical applicability in serum. Conceptually, it validates a paradigm shift away from the "one test, one disease" model toward a more holistic, multiplexed approach to health monitoring,

providing a tangible technological pathway to the future of personalized and preventative medicine.

This study's conclusions are framed by its validation using spiked serum samples, which, while robust, precedes full clinical trials and thus defines the trajectory for future research. The most critical next steps must involve a large-scale clinical validation study with real patient samples to determine diagnostic accuracy and establish clinical cutoff values. Subsequent research should focus on expanding the biomarker panel for other disease states, integrating the sensor chip into a fully automated point-of-care device, and optimizing the manufacturing processes for scalable and cost-effective production.

AUTHOR CONTRIBUTIONS

Look this example below:

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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