Journal of Biological Engineering

REVIEW Open Access

# Nanozyme-based aptasensors for the detection of tumor biomarkers

Ke Wang[1], Xiaogang Lin[1*], Xiao Lv[1], Mingna Xie[1], Jinyu Wu[1], Jie Jayne Wu[2*] and Yang Luo[3]

**Abstract**

A nanozyme-based aptasensor combines the unique properties of nanozymes with the specificity of aptamers for the detection of various biomolecules. Nanozymes are nanomaterials that possess enzyme-like properties, demonstrating substantial potential for enhancing the sensing capabilities of biosensors. In recent years, the incorporation of nanozymes into biosensors has opened new avenues for the detection of tumor biomarkers. The unique attributes of nanozymes and aptamers lead to biosensors characterized by high sensitivity, specificity, reproducibility and accuracy in analytical performance. This article reviews the research progress of nanozyme-based aptasensors in tumor biomarker detection over the past decade. We categorize these sensors based on their sensing modes and target types, and examine the properties and applications of the nanozymes employed in these devices, providing a thorough discussion of the strengths and weaknesses associated with each sensor type. Finally, the review highlights the strengths and challenges associated with nanozyme-based biosensors and envisions future developments and applications in this field. The objective is to provide insights for improving biosensor performance in tumor biomarker detection, thereby contributing to advancements in precision cancer diagnosis and treatment.

**Keywords** Nanozymes, Aptamers, Tumor biomarkers, Biosensors, Precision diagnostic

## Introduction

Tumor biomarkers are active substances associated with the presence and progression of cancer, and their accurate quantification holds significant clinical value [1]. Liquid biopsy is a non-invasive method that detects tumor biomarkers in bodily fluids such as blood, saliva, and urine, providing a robust foundation for early diagnosis, personalized treatment monitoring, and prognosis assessment. Key targets in liquid biopsy include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), extracellular vesicles (EVs), and circulating tumor RNA (ctRNA) [2–5]. Among these tumor markers, the EpCAM marker on the surface of circulating tumor cells (CTCs), analyzed using immunomagnetic capture and immunofluorescence, as well as carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA) assessed through immunoassays, have received approval from the Food and Drug Administration (FDA) for clinical use [6]. Currently, blood-based liquid biopsies are the primary focus of research. Traditional methods for detecting tumor biomarkers include enzyme-linked immunosorbent assay (ELISA) [7], real-time polymerase chain reaction (Real-time PCR) [8], spectroscopic techniques [9], and flow cytometry [10]. However, the clinical

*Correspondence:
Xiaogang Lin
xglin@cqu.edu.cn
Jie Jayne Wu
jaynewu@utk.edu
[1]Key Laboratory of Optoelectronic Technology and Systems of Ministry of Education of China, Chongqing University, Chongqing 400044, China
[2]Department of Electrical Engineering and Computer Science, The University of Tennessee, Knoxville, TN 37996, USA
[3]Chongqing General Hospital, Chongqing University, Chongqing 401147, China

applications of these methods face significant limitations due to low sensitivity, complex and time-consuming procedures, the need for large sample sizes, and high costs [11]. Therefore, there is an urgent need to develop rapid, sensitive, and cost-effective detection methods to facilitate early cancer diagnosis in a convenient and timely manner.

Enzyme-based immunoassay is considered to be one of the most widely used detection techniques, and has been applied in the fields of environmental monitoring, food safety, and medical diagnosis [12–14]. Natural enzymes play a crucial role in the development of these assays due to their unique biocatalytic activity. However, natural enzymes have several limitations, including high cost, poor stability, difficulties in long-term storage and mass production, and challenges in withstanding harsh environments. Moreover, the detection performance of immunoassays heavily relies on antibodies, whose activity depends on the immune response [15]. Antibodies are large molecules, costly, difficult to modify at specific sites, and prone to significant batch-to-batch variability, especially in polyclonal antibodies obtained from animal blood [16]. As a result, the sensitivity of conventional enzyme-based immunoassays is often insufficient for the ultrasensitive detection of trace biomolecules.

Nanomaterials offer several advantages, including a high surface area-to-volume ratio, an improved ability to immobilize biomolecules, and enhanced capabilities for signal generation, amplification, and transduction [17]. Rahmanian et al. [18] realized electrochemical and fluorescence dual detection of aflatoxin B1 (AFB1) using $Fe_3O_4$@AuNPs/ZIF-8 nanocomposites with a large amount of gold surface and aptamer probes. In 2013, Wei et al. [19] united nanomaterials with enzyme-like activities under the name of nanozymes. In recent years, nanozyme aptasensors have been developed, mimicking the principles of enzyme-based immunoassay in terms of target detection and signal amplification. offer several advantages over natural enzymes, including enhanced stability, lower production costs, and the potential for tailored design to improve their catalytic activity, effectively addressing the limitations associated with natural enzymes [20]. Furthermore, nanozymes exploit the unique properties of nanostructures, such as high conductivity and an expansive surface area, which contribute to their efficiency in catalyzing biochemical reactions [21]. Nanozymes can act as recognition receptors or signaling tags. In addition, they can be used for signal amplification in combination with various sensing technologies such as electrochemical [22], fluorescence [23], colorimetric [24], and photoelectrochemical [25] methods. These technologies enable the detection of ions, nucleic acids, proteins, and cancer cells [26]. Electrochemical sensing has the advantages of low cost, simplicity, time saving and easy execution. Colorimetric sensors have attracted more attention due to their low cost, simple operation and short detection time. Fluorescence methods only require simple equipment such as a handheld fluorimeter or fluorescence spectrometer to achieve accurate and sensitive detection of the target. Dadmehr et al. [27] developed a colorimetric/fluorescence dual strategy for sensitive detection of AFB1 based on multiple aptamer-mediated probes and nanozyme activity. This platform has the advantages of sensitive fluorescence analysis and nanozyme-assisted colorimetry, achieving high-sensitivity detection of AFB1. Photoelectrochemical (PEC) sensing uses light as the excitation source and the generated photocurrent as the detection signal. This technology has low background signal, excellent detection performance and simple operation. Based on the types of reactions they catalyze, nanozymes can be classified as oxidase (OXD) mimics, peroxidase (POD) mimics, catalase (CAT) mimics, superoxide dismutase (SOD) mimics, and hydrolase mimics. Some nanomaterials can simultaneously exhibit multiple enzyme-mimicking activities and have broad application prospects in various fields such as medical diagnosis, chemical engineering, food, agriculture and environmental science [28].

The recognition elements of tumor markers usually include antibodies, aptamers, and peptides [29]. Compared with more expensive antibodies and complex peptides, aptamers with stronger target binding ability can be obtained through in vitro screening and amplification steps [30]. Aptamers are short single-stranded DNA or RNA molecules with high specificity and affinity for targets and are called “chemical antibodies” [31]. The structure of aptamers is relatively flexible and can be easily modified chemically. Due to their multiple functions, such as targeted binding, signal amplification, and modification of the catalytic properties of nanomaterials, aptamers have a wide range of applications in medical diagnosis and treatment [32–34]. One of the most common applications is to bind nucleic acid aptamers to target molecules for early disease detection and diagnosis.

The combination of nanozymes and aptamers has led to the development of numerous nanozyme-based aptasensors. Aptamers provide high specificity for target recognition, while nanozymes are responsible for signal generation [35]. In addition, interactions between some aptamers and nanozymes can either inhibit or enhance the activity of nanozymes when aptamers are modified on nanozymes [36, 37]. Recent research advances have highlighted the potential applications of nanozyme-based aptasensors for tumor biomarker detection. This paper reviews the research progress of nanozyme-based aptamer biosensors in early cancer diagnosis, metastasis monitoring and treatment evaluation in the last decade.

Figure 1 summarizes nanozyme-based aptamer biosensing strategies for the detection of tumor biomarkers. The biosensors discussed in this paper mainly utilize peroxidase-like and catalase-like activities. The relevant studies are categorized according to sensing modalities and target types. The review examines various nanozymes, their catalytic properties, signal transduction strategies, detection times, and the efficacy of nanozyme-based biosensors in detecting CTCs, exosomes, and other protein-based tumor biomarkers. The advantages and limitations of these sensors are summarized, along with their potential in clinical diagnosis and personalized medicine. Finally, the review outlines future prospects for nanozyme-based aptamer biosensors, including enhancements in nanozyme catalytic activity, innovative sensor design and integration, and the expansion into new application areas through interdisciplinary collaborations. This review aims to inform the development of precision cancer diagnosis and treatment.

## Single-mode sensing strategies

A single-mode nanozyme aptasensor refers to a biosensor that operates using a single detection mechanism to identify and quantify a target biomolecule. Currently, single-mode sensing strategies for the detection of tumor biomarkers include electrochemical, optical, photothermal, and photoelectrochemical. Electrochemical detection is fast and convenient with high sensitivity for on-site detection [38]. Commonly used electrochemical measurement methods include cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPV). Optical methods are easy to manipulate and resistant to interference [39]. Commonly used optical measurement methods include colorimetric, fluorescence, and surface enhanced Raman scattering (SERS). Photothermal detection is low cost, simple to operate, and easy to realize in situ measurement [40]. Photoelectrochemistry effectively combines photochemical technology with electrochemistry, which not only inherits the advantages of traditional electrochemical methods, but also improves sensitivity and reduces background interference due to the effective separation of detection signals and excitation sources [41]. Each of these sensing strategies has its own advantages and has a wide range of applications in cancer diagnosis and treatment.

### Detection of circulating tumor cells

CTCs are a rare and heterogeneous type of cells found in the peripheral blood of cancer patients. They are considered a central component of metastatic dissemination and are associated with prognosis, progression-free survival, and treatment efficacy in different solid tumors [42]. CTCs are released into the bloodstream from primary tumors or metastatic circulating cancer cells that may display overall tumor characteristics, and are used as markers reflecting biological and clinical aspects of liquid biopsy markers, thus facilitating non-invasive real-time monitoring of cancer patients [43]. Direct detection and analysis of CTCs in peripheral blood is a non-destructive and safe liquid biopsy method compared to tumor tissue

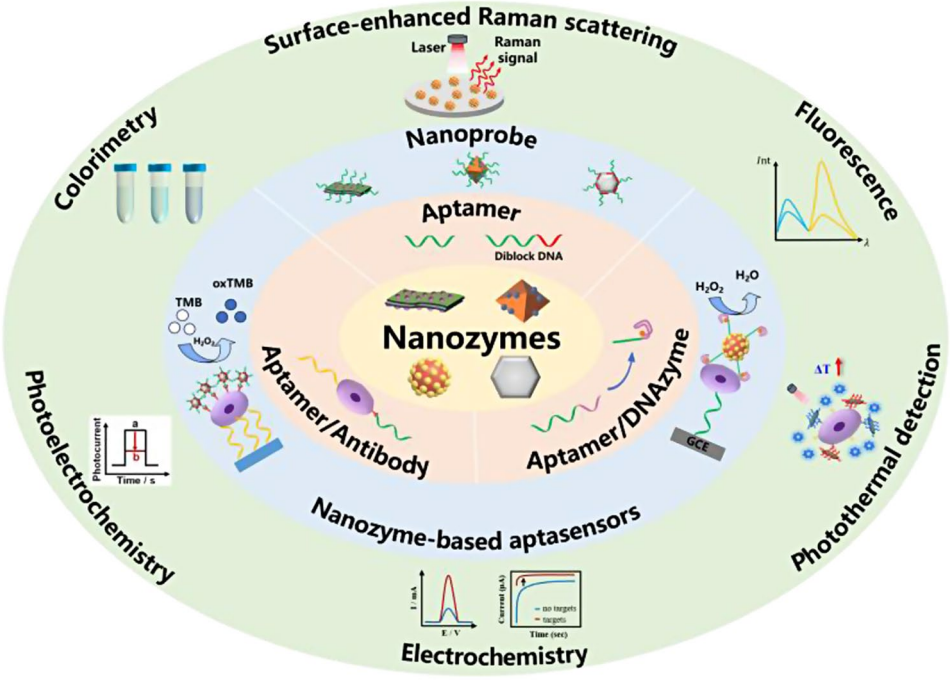


**Fig. 1** Schematic diagram of the strategy to construct aptamer biosensors using nanozymes for the detection of tumor biomarkers

biopsy. CTCs are rarely distributed in peripheral blood, so they need to be separated from blood cells and further purified. Traditional separation methods usually include density gradient centrifugation, filtration, and immunomagnetic separation [44]. Among them, immunomagnetic separation is currently the most commonly used CTCs separation method. In recent years, research on CTCs separation and detection using microfluidic chips has progressed rapidly, and a variety of technical principles and detection methods have emerged one after another [45]. The methods for identifying CTCs mainly include immunofluorescence [46], fluorescence in situ hybridization [47], reverse transcription polymerase chain reaction (RT-PCR) [48], Real-time PCR (RT-PCR) [49], and flow cytometry [50]. Although these methods are mature and well established, most of the techniques for detecting CTCs are time-consuming and require skilled operators and expensive instruments. Aptamers are single-stranded oligonucleotides with good affinity and specificity, which bind to targets on the surface of CTCs by folding into a unique tertiary structure. The combination of aptamers and nanozymes with excellent catalytic properties provides a new method to improve the detection performance of CTCs. Therefore, this section will comprehensively summarize the relevant research progress of simple and highly sensitive nanozyme aptamer biosensors for detecting CTCs based on electrochemical, photothermal, colorimetric, fluorescence, SERS, and other sensing technologies, discuss their potential application in the detection of real human blood samples, and compare the detection performance of each sensor in Table 1.

Breast cancer (BC) is one of the most serious diseases threatening women's health, and circulating tumor cells are a reliable biomarker for non-invasive early breast cancer diagnosis. Tian et al. [51] developed an ultrasensitive electrochemical assay for the detection of CTCs using reduced graphene oxide/gold nanoparticle composites (rGO/AuNPs) as a carrier and CuO nanozymes as catalysts (Fig. 2A). In this study, CuO nanozymes were used as signal amplification nanoprobes for ultrasensitive detection of MCF-7 CTCs for the first time. Due to the high specific surface area, fast electron transport, and good biocompatibility of rGO/AuNPs composites, the mucin 1 (MUC-1) aptamer immobilized on its surface has high stability and bioactivity, and can specifically recognize MCF-7 CTCs. The amplified signal of the CuO nanozyme/$H_2O_2$ system can be sensitively detected by DPV, and the linear detection range of the proposed cell sensor for MCF-7 CTCs is $50-7\times10^3$ cells/mL, with a detection limit as low as 27 cells/mL. CuO nanozymes with peroxidase mimetic activity and excellent catalytic performance for reducing $H_2O_2$ can significantly improve the detection performance of electrochemical cell sensors. In the same year, Tian et al. [52] adopted the rGO/$MoS_2$ and $Fe_3O_4$ NPs dual nanozyme synergistic signal amplification strategy to construct another electrochemical cell sensor for ultrasensitive detection of MCF-7 CTCs. The principle of the sensor is shown in Fig. 2B. The MUC-1 aptamer-modified $Fe_3O_4$ NPs magnetic beads are fixed on the surface of the magnetic glassy carbon electrode (MGCE) by inserting a magnet. The cell sensor can be regenerated by simply pulling out the magnet. The amplified signal of the TMB/$H_2O_2$ system catalyzed by dual nanozymes was detected using DPV. The linear detection range of the proposed cell sensor is 15 to 45 cells/mL, and MCF-7 CTCs as low as 6 cells/mL can be detected. The rGO/$MoS_2$ composite material and $Fe_3O_4$ NPs show good electrocatalytic activity in synergistic catalysis, thereby improving the stability and sensitivity of

**Table 1** Single-mode aptamer biosensors based on nanozymes for circulating tumor cell detection

| Cell Line | Aptamer | Nanozyme | Substrate | Detection Method | Electrode | Linear Range | LOD | Recovery (RSD) | Assay Time | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| MCF-7 | MUC-1 | CuO | $H_2O_2$ | DPV | GCE | $50-7\times10^3$ cells/mL | 27 cells/mL | 92 – 105.8% (< 4.25%) | – | [51] |
| | MUC-1 | rGO/$MoS_2$ $Fe_3O_4$ | $H_2O_2$ | DPV | MGCE | 15 – 45 cells/mL | 6 cells/mL | 88.9 – 108.6% (< 5.69%) | 60 min | [52] |
| | EpCAM | Au@PdMo | $H_2O_2$ | DPV | GCE | $2-1\times10^5$ cells/mL | 2 cells/mL | 97.78–104.20% (1.11–2.85%) | – | [53] |
| | MUC-1 $VEGF_{165}$ EpCAM | MXene@Au@Pt | TMB/$H_2O_2$ | Photothermal analysis | – | $50-1.0\times10^5$ cells/mL | 50 cells/mL | – | – | [54] |
| CEM | sgc8c | h-JHNzyme | 4-MPB/$H_2O_2$ | SERS | – | 10 – 10,000 cells | 1 cell | 95.7% (5.8%) | – | [55] |
| HepG2 | AS1411 | Fe-N-C SAzymes | TMB/$H_2O_2$ | Colorimetry | – | $100-2\times10^5$ cells | – | – | – | [56] |
| | TLS11a | $Fe_3O_4$@Au | $H_2O_2$ | Chronoamperometry | GCE | $1\times10^2$–$\times10^7$ cells/mL | 20 cells/mL | 85.3 – 91.1% (5.13 – 6.56%) | – | [57] |
| HeLa | AS1411 | UIO-66(Fe/Zr) | TMB/$H_2O_2$ | Colorimetry | – | $10^3$–$10^4$ cells/mL | 481 cells/mL | 96.8 – 106.0% (2.3 – 4.7%) | – | [59] |

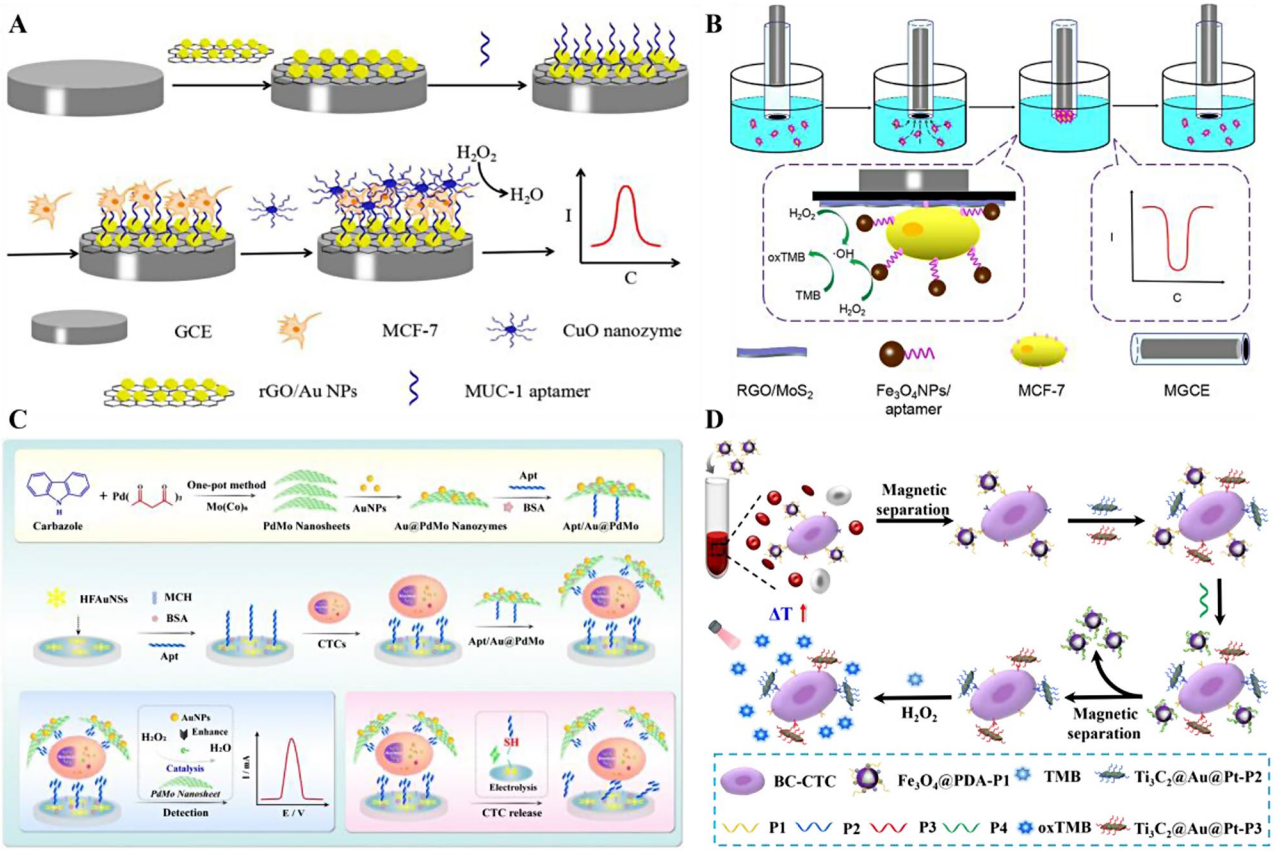


**Fig. 2** Aptasensors for the detection of MCF-7 CTCs. (**A**) Schematic diagram of the cell sensor and detection principle. Reprinted with permission from [51]. (**B**) Schematic diagram of the magnetic bead-assisted dual nanozymatic signal amplification method for CTC detection. Reprinted with permission from [52]. (**C**) The preparation process of Apt/Au@PbMo nanozyme signal amplification probe, the detection principle of cell sensor, the electrochemical signal generated by Au@PdMo nanozyme catalyzing $H_2O_2$, and the electrochemical reduction desorption mechanism of releasing CTCs. Reprinted with permission from [53]. (**D**) Principle of the portable photothermal cell sensor for direct capture and quantification of CTCs. Adapted with permission from [54]

the biosensor. Yang et al. [53] combined two-dimensional Au@PdMo nanozymes with electrochemical reduction desorption to construct a new cell sensor for ultrasensitive detection and non-destructive release of MCF-7 CTCs. The schematic diagram of the sensor is shown in Fig. 2C. Apt/Au@PdMo signal amplification probe is prepared in advance, and HFAuNSs composite material and EpCAM aptamer are modified on the surface of glassy carbon electrode (GCE). When MCF-7 CTCs are present, they are specifically recognized by EpCAM aptamer. At this time, the signal amplification probe is added to form Apt/CTC/Apt/Au@PdMo bioconjugate on the electrode surface. The peroxidase-like activity of Au@PdMo nanozymes is used to achieve significant DPV signals. The linear range of the cell sensor is $2-1\times10^5$ cells/mL, and the detection limit is 2 cells/mL. Under electrochemical reduction desorption, the captured CTCs can be released from the electrode without damage. Yang et al. [54] also proposed a portable photothermal cell sensor for high-sensitivity detection of MCF-7 CTCs. The detection principle of the sensor is shown in Fig. 2D. The sensor uses MUC-1 aptamer to capture MCF-7 CTCs. In addition, two multifunctional $Ti_3C_2$@Au@Pt nanozymes were prepared based on Vascular endothelial growth factor 165 ($VEGF_{165}$) aptamer and EpCAM aptamer. $Ti_3C_2$@Au@Pt not only has excellent photothermal effect, but also exhibits high peroxidase-like activity. The catalytic activity of Ti3C2@Au@Pt NCs was verified by the colorimetric effect. It can catalyze 3,3',5,5'-tetramethylbenzidine (TMB) to generate oxidized TMB (oxTMB) with strong photothermal properties, and the combination of oxTMB and $Ti_3C_2$@Au@Pt can amplify the temperature signal. In the range of $50-1.0\times10^5$ cells/mL, the logarithmic curve of ΔT and MCF-7 CTCs concentration showed a good linear relationship, and the sensor reached a detection limit of 50 cells/mL. The results showed that the application of nanozymes significantly improved the sensitivity of MCF-7 CTCs aptasensors, and the sensitivity of the electrochemical sensor was relatively higher.

Acute lymphoblastic leukemia (ALL) is one of the most common types of malignant tumors in the circulatory system, and leukemic cell CEM can be used for

early diagnosis of ALL. Su et al. [55] designed a spatially engineered hollow Janus hybrid nanozyme (h-JHNzyme) based on bidirectional modulation of Ag-Au nanocages, and used h-JHNzyme as a catalytic label for SERS liquid biopsy. The quantitative analysis of human acute lymphoblastic leukemia-related CEM cells and miRNA-21 was successfully achieved through a ratiometric SERS detection strategy. This study mainly analyzes its application in CEM cell detection. Figure 3A shows the preparation process of h-JHNzyme and the principle of SERS detection using h-JHNzyme. The tandem DNAzyme nanostructure was in situ assembled on the surface of the nanozyme by cascade hybridization chain reaction (HCR). The internal silver gates and external nanobrushes of DNAzyme molecules endowed h-JHNzyme with excellent peroxidase mimicry activity, good biocompatibility, high stability, and specific targeting functionality. The CEM aptamer "HS-sgc8c" was modified on the surface of the gold electrode by Au-S bonding. When CEM cells were present, CEM cells were captured at the electrode by specific recognition of aptamers. The number of h-JHNzymes at this time can reflect the number of CEM cells by inhibiting the overall catalytic activity of $H_2O_2$ oxidation of 4-mercaptophenylboronic acid (4-MPB) and output by the proportional SERS strategy. In the range of 10 – 10,000 CEM cells, the ratio IR increases linearly with the logarithm of the number of CEM cells, and the detection limit is only 1 cell. This study demonstrates that h-JHNzyme is a powerful "all-in-one" enzyme carrier with excellent biocompatibility, specificity vectorization, significant enzymatic properties and clinical utility.

Hepatocellular carcinoma is one of the deadliest malignant tumors in the world, and quantitative detection of human hepatocellular carcinoma HepG2 cells has received much attention in recent years. Sun et al. [56] designed an aptamer-modified Fe-N-C SAzymes (Apt/Fe-N-C SAzymes) for colorimetric detection of HepG2 cells. As shown in Fig. 3B, diblock DNA modified by a polyadenine block and an AS1411 aptamer block formed Apt/Fe-N-C SAzymes on the surface of Fe-N-C SAzymes, which targeted HepG2 cells by recognizing nucleolin through the AS1411 aptamer. The linear range of Apt/Fe-N-C SAzymes for detecting HepG2 tumor cells was $100 - 2 \times 10^5$ cells. The developed cancer cell biosensor combines the peroxidase-like catalytic activity of Fe-N-C SAzymes with the high specificity of cell-specific aptamers. Inspired by this strategy, other SAzymes and molecular recognition elements can be introduced into a universal bioanalytical format for clinical diagnosis and treatment. Liu et al. [57] constructed an electrochemical cytosensor with triple signal amplification based on $Fe_3O_4$@Au nanozyme and DNAzyme hybrids, an electrochemical cytosensor with triple signal amplification was constructed for sensitive detection of HepG2 cells. $Fe_3O_4$@Au nanozyme has peroxidase-like activity, which not only catalyzes $H_2O_2$ reduction, but also serves as a scaffold for carrying a large number of electroactive substances and DNA probes. Therefore, toluidine blue (Tb) was modified onto the surface of $Fe_3O_4$@Au nanohybrids to amplify the electrochemical response as an electron transport medium. In this study, hemin/G-quadruplex was used as DNAzyme and TLS11a aptamer to constitute a DNA probe with dual functions of signal amplification and recognition to detect HepG2 cells. The cell sensor achieved sensitive detection of HepG2 cells in a linear range of $1 \times 10^2 – 1 \times 10^7$ cells/mL with a detection limit as low as 20 cells/mL. This electrochemical cell sensor is expected to be an effective analytical tool for early cancer diagnosis in the future.

As a gynecological malignancy, cervical cancer occurs mostly in young patients [58]. In order to improve the prognosis of cervical cancer patients and achieve effective diagnosis of early cervical cancer, Zhu et al. [59] proposed a novel single aptamer-dependent sandwich-type biosensor for the colorimetric analysis of cancer cells. The sensor uses $Fe_3O_4$@$SiO_2$-AS1411 Apt to selectively

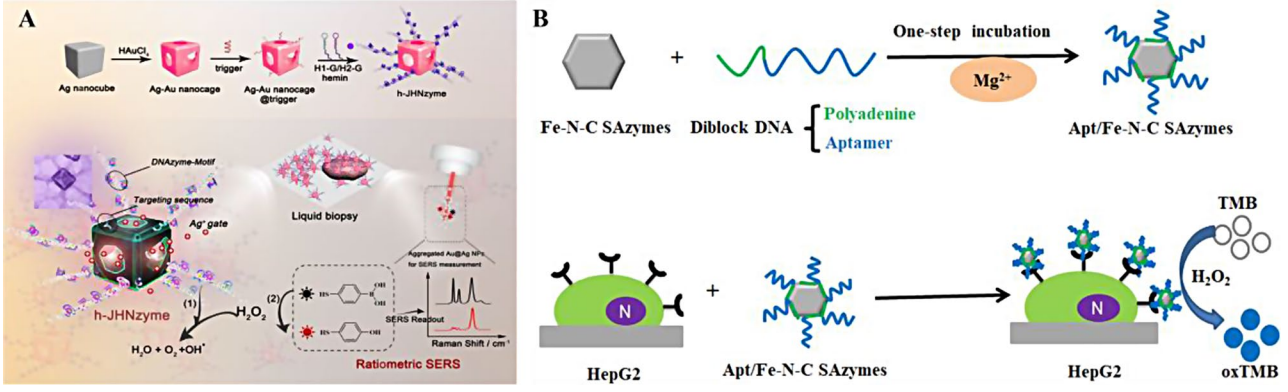


**Fig. 3** (**A**) The preparation process of h-JHNzyme and the principle of SERS detection using h-JHNzyme. Reprinted with permission from [55]. (**B**) The preparation process of Apt/Fe-N-C SAzymes and the principle of colorimetric detection of cancer cells. Reprinted with permission from [56]

capture and magnetically separate target cancer cells and UIO-66 (Fe/Zr) nanozymes to catalyze the TMB/$H_2O_2$ system to amplify the colorimetric signals for simple, sensitive, and specific isolation and detection of HeLa cells. UIO-66 Metal-Organic Frames (MOFs) have a favorable crystal structure, formed by introducing Fe/Zr bimetallic nodes to form UIO-66(Fe/Zr) nanozymes, which provide peroxidase-like nanozymatic activity and are able to bind directly to the cell surface based on the ligand bonding involving Zr. This novel sandwich-type aptasensor achieved a simple, sensitive and specific detection of HeLa cancer cells with a linear range of $10^3$–$10^4$ cells/mL and a detection limit of 481 cells/mL. This novel single aptamer colorimetric sandwich-type biosensor has a great potential in the diagnosis and therapeutic evaluation of cancer.

#### Detection of exosomes

Exosomes are nanoscale extracellular vesicles (30–150 nm) with a lipid bilayer membrane structure and are commonly found in all body fluids (blood, saliva, urine, etc.) and cell lines cultured in vitro [60]. Exosomes carry biological molecules such as proteins, lipids, and nucleic acids related to the parent cells. Therefore, tumor-derived exosomes can reflect the dynamic changes and development of tumors [61]. In addition, exosomes have a stable lipid bilayer and can be used as specimens for long-term storage. Compared with CTCs, tumor-derived exosomes are abundant in the blood, with a concentration of up to $10^9$ particles/mL. A large number of studies have shown that the level of exosomes in the systemic circulation of cancer patients is significantly increased. Exosomes can usually be obtained from cell culture supernatants or blood, and this acquisition method is non-invasive. Therefore, exosomes have good application prospects as non-invasive diagnostic markers for cancer. At present, the methods for isolating exosomes mainly rely on differences in particle size or specific surface markers to extract exosomes, including ultracentrifugation, size exclusion chromatography, and microfluidics. Ultracentrifugation is the current "gold standard" for exosome isolation, but this method relies on expensive instruments, consumes a lot of samples, is time-consuming and labor-intensive to operate, and has a low exosome yield [62]. Size exclusion chromatography can separate exosomes from soluble proteins, but the sample must be pretreated by ultracentrifugation or ultrafiltration. In addition, the exosomes on the separation column require a large amount of buffer solution to elute, resulting in a relatively low concentration of collected exosomes. The development of microfluidics has solved the problems of long time and high cost of exosome separation, and has become a hot topic in exosome separation research. The characterization and detection of exosomes after separation helps to identify the morphology, quantity and level of exosome-specific molecular markers, which plays an important role in the early diagnosis, treatment and prognosis of diseases. In recent years, quantitative studies of exosomes based on proteins on the exosome membrane have attracted widespread attention [63]. To date, there have been several assays for the detection of protein expression on the surface of exosomes, including Western blotting (WB) [64] and ELISA [65], etc. However, these methods often have the disadvantages of time-consuming, large sample size, and cumbersome preparation process. In addition, the low abundance and complex composition of tumor-associated exosomes in biological samples hinder the clinical application of exosome-based cancer diagnosis [66]. Therefore, this section will comprehensively summarize the relevant research advances of highly sensitive and specific nanozyme-based single-mode aptamer biosensors for the detection of protein expression on exosome surfaces, discusses the potential of their application for the detection of real human blood samples, and compares the assay performance of individual sensors in Table 2.

To further improve the sensitivity of fluorescence detection of exosomes, Liu et al. [63] constructed a nanozyme sensor array using aptamer-modified $C_3N_4$ nanosheets (Apt/$C_3N_4$ NSs) and employed a 1,4-dioxane-solvent mediated signal amplification strategy for ratiometric fluorescence detection of exosomal proteins (Fig. 4A). In this study, three specific exosomal proteins MUC-1, EpCAM, and human epidermal growth factor receptor 2 (HER2) aptamers were used to construct Apt/$C_3N_4$ NSs to achieve highly specific recognition of exosomal proteins. The construction of heterodimers of $C_3N_4$ NSs with peroxidase-like activity and aptamers

**Table 2** Single-mode aptamer biosensors based on nanozymes for cancer cell-derived exosomes detection

| Cell Line | Aptamer | Nanozyme | Substrate | Detection Method | Linear Range | LOD | Recovery (RSD) | Assay Time | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| MGC-803 | MUC-1 EpCAM HER2 | $C_3N_4$ nanosheet | oPD/$H_2O_2$ | Fluorescence | $8.53\times10^3$–$3.01\times10^6$ particles/mL | $2.5\times10^3$ particles/mL | – | – | [63] |
| HGC-7910 | CD63 | $MoS_2$ -MIL-101(Fe) | TMB/$H_2O_2$ | Colorimetry | $1.6\times10^4$–$1.6\times10^6$ particles/μL | $3.37\times10^3$ particles/μL | 95 – 103% | – | [36] |
| MCF-7 | CD63 | $Fe_3O_4$-$Cu^{2+}$ | TMB/$H_2O_2$ | Colorimetry | $1.4\times10^4$–$5.6\times10^5$ particles/μL | $5.91\times10^3$ particles/μL | – | – | [37] |

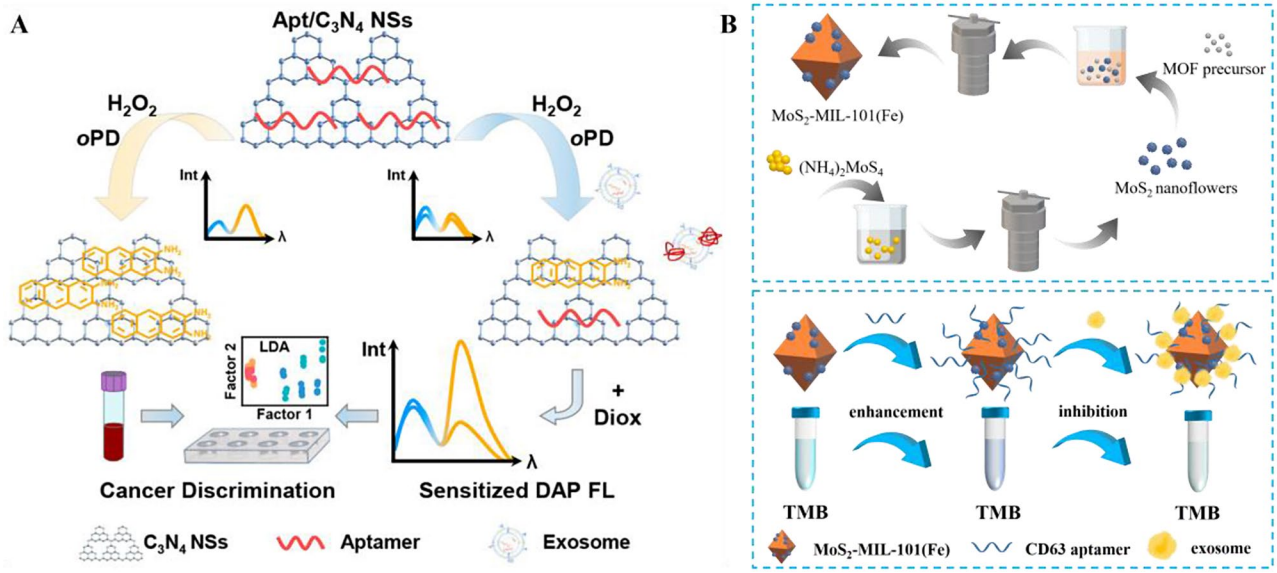


**Fig. 4** **A** Nanozyme sensor array plus solvent-mediated signal amplification strategy for ultrasensitive detection of exosomal proteins and cancer identification. Reprinted with permission from [63]. **B** The synthesis process of $MoS_2$-MIL-101(Fe) and schematic diagram of exosome detection mechanism of the proposed method. Adapted with permission from [36]

enhances the efficiency of catalyzing the oxidation of o-phenylenediamine (oPD) to the fluorescent molecule 2,3-diaminophenazine (DAP). When the target exosome is present, the strong affinity between the aptamer and exosome disassembles the Apt/$C_3N_4$ NSs, leading to a decrease in the catalytic activity of the $C_3N_4$ NSs nanozymes, which reduces the production of DAP. The nanozyme sensor array had a detection limit of $2.5 \times 10^3$ particles/mL for MUC1-positive exosomes from MGC-803 cells over a linear range of $8.53 \times 10^3$–$3.01 \times 10^6$ particles/mL. In addition, in this study, the detection limit of MUC1-positive exosomes from MGC-803 cells was determined by a linear discriminant analysis (LDA) algorithm recognized exosomes secreted by five cell types with 100% accuracy and was able to completely separate gastric cancer patients from healthy volunteers by differences in exosomal proteins in the blood.

CD63 is a highly N-glycosylated type III lysosomal membrane protein known to regulate malignant tumors such as melanoma and breast cancer [67]. Li et al. [36] developed a visual and simple exosome colorimetric detection method based on molybdenum disulfide nanoflowers modified iron organic framework ($MoS_2$-MIL-101(Fe)) hybrid nanozyme and CD63 aptamer. Figure 4B shows the preparation method of $MoS_2$-MIL-101(Fe) nanozyme and the principle of exosome detection. The structure of MIL-101(Fe) gives $MoS_2$ a large specific surface area, which is conducive to modifying probes on the outside of the hybridized nanozymes. The CD63 aptamer modified on the surface of the hybridized nanozymes not only specifically recognizes and captures tumor exosomes, enhances the peroxidase activity of the hybridized nanozymes, and helps to catalyze the TMB-$H_2O_2$ system to produce a stronger colorimetric signal. When exosomes are present, exosomes are recognized by the CD63 aptamer and adsorbed on the surface of the nanozymes, restoring the enhanced peroxidase activity of $MoS_2$-MIL-101(Fe)@aptamer, resulting in a dark to medium color change in the TMB-$H_2O_2$ system, which can be visibly visualized and measured by the UV-vis spectrometer. The linear range of the exosome assay proposed in this study was $1.6 \times 10^4$–$1.6 \times 10^6$ particles/μL, and the limit of detection was $3.37 \times 10^3$ particles/μL. The nanozymes-based colorimetric aptasensor constructed in this study provides insights into the measurement of novel tumor biomarkers in the field of biosensors. However, the defects of relatively low substrate selectivity and catalytic efficiency of the nanozymes have not been resolved, hindering further improvement of the sensitivity of this aptasensor.

Long et al. [37] designed a colorimetric aptasensor for breast cancer exosome detection based on iron-copper oxide-copper ion nanozymes ($Fe_3O_4$-$Cu^{2+}$-NZs) and CD63 aptamers. In this study, CD63 aptamers were modified on the surface of $Fe_3O_4$-$Cu^{2+}$-NZs based on a hydrothermal method, and the performance of CD63 aptamer-$Fe_3O_4$-$Cu^{2+}$-NZs in catalyzing the oxidation of TMB was evaluated by UV-vis spectroscopy as well as by naked eye observation. The results showed that the adsorption of CD63 aptamer resulted in a significant inhibition of the peroxidase-like activity of $Fe_3O_4$-$Cu^{2+}$-NZs. When exosomes were present, the peroxidase-like activity of $Fe_3O_4$-$Cu^{2+}$-NZs was enhanced due to the specific recognition of exosomes with CD63 aptamer,

resulting in the restriction of exosomes to the surface of CD63 aptamer-$Fe_3O_4$-$Cu^{2+}$-NZs. The absorbance of the UV-Vis spectrum at 625 nm decreased as the concentration of exosomes increased. The CD63 aptamer-$Fe_3O_4$-$Cu^{2+}$-NZs designed in this study was able to detect exosomes in a linear range of $1.4 \times 10^4$–$5.6 \times 10^5$ particles/μL, with a limit of detection of $5.91 \times 10^3$ particles/μL. The use of CD63 aptamer-$Fe_3O_4$-$Cu^{2+}$-NZs as a colorimetric diagnostic method for breast cancer has the advantages of being label-free, easy to fabricate, highly accurate, and easy to use in real samples. However, the preparation process of nanozymes is complicated and has the potential to produce non-specific binding, which can easily lead to system instability.

#### Detection of carcinoembryonic antigen

Carcinoembryonic antigen (CEA) is one of the most common tumor biomarkers in clinical practice and is used for screening, diagnosis, and prognostic evaluation of many gastrointestinal tumors, including colorectal, esophageal, gastric, and pancreatic cancers [68]. Therefore, rapid and sensitive detection of CEA is very important for cancer diagnosis and treatment. Currently, the most classical CEA detection method is enzyme-linked immunosorbent assay (ELISA), which relies on enzyme catalysis and antigen-antibody recognition [69]. However, the complexity of its operational steps and low detection accuracy have hindered the development of immunosensor detection methods. In addition, anti-CEA antibodies for immunoassays are relatively costly and labile in nature. Aptamers, as promising alternatives to antibodies, have the advantages of good stability, easy synthesis and modification, low cost, fast tissue penetration, and low toxicity, which, combined with the strong stability and efficient catalytic properties of nanozymes, are extremely attractive for the construction of biosensors. In this section, relevant research advances on easy-to-construct, sensitive and stable, and cost-effective nano-enzyme-based single-mode aptamer biosensors for the detection of CEA are comprehensively summarized to explore their potential applications in the detection of real human blood samples, and the detection performance of individual sensors is compared (Table 3).

Zhao et al. [70] developed a colorimetric sensing platform for rapid and sensitive detection of CEA based on DNA-modulated $MoS_2$ NS nanozymes. In the absence of CEA, the enhanced catalytic activity was mainly due to the increased affinity of the DNA aptamer-modified $MoS_2$ NSs for the peroxidase substrate TMB. In the presence of CEA, the CEA aptamer preferentially binds to CEA and releases it from the surface of $MoS_2$ NSs, leading to a decrease in the catalytic activity of $MoS_2$ NSs. At this time, the UV-vis absorbance of the TMB-$H_2O_2$ system decreased, and the color of the solution became lighter. The CEA concentration and oxTMB absorbance showed a linear relationship in the range of 50 – 1,000 ng/mL, with a detection limit of 50 ng/mL. This novel sensing platform is not only simple in design, but also does not involve oligonucleotide labeling or complex nanomaterial modification processes.

Chen et al. [71] designed a simple voltammetric sensor based on aptamer control of the catalytic activity of Pt NPs/Fe-MOF nanozymes, and selected CEA aptamer as the specific recognition element to capture CEA. Fe-MOF loaded with platinum nanoparticles (Pt NPs/Fe-MOF) was prepared by a simple hydrothermal method, and the synthesized Pt NPs/Fe-MOF nanozymes effectively catalyzed the oxidation of oPD to generate electroactive DAP with a strong reducing current. The catalytic activity was inhibited when CEA aptamer was modified on the surface of Pt NPs/Fe-MOF nanozymes, and reinstated in the presence of CEA. In addition, the peak current of DAP increased gradually with the increase of CEA concentration. The absolute value of the change in the peak current of DAP in the presence and absence of CEA ($\Delta I_p$) was selected as the correlation output signal, and there was a linear relationship between the value of $\Delta I_p$ and the concentration of CEA in the range of 0.003 – 15.0 ng/mL, with a limit of detection of 1.0 pg/mL. This nanozyme-based voltammetric sensor has a better performance for the detection of CEA in the realizing simple, sensitive and efficient CEA determination with great potential.

#### Detection of other protein tumor biomarkers

In addition to the above-mentioned tumor biomarkers such as circulating tumor cells, exosomes, and carcinoembryonic antigen, which are present in a wide range of cancers, there are also some specific protein tumor biomarkers that are present in only a few cancers. In this section, we will comprehensively summarize the research progress related to the sensitive and low-cost

**Table 3** Single-mode aptamer biosensors based on nanozymes for carcinoembryonic antigen detection

| Biomarker | Aptamer | Nanozyme | Substrate | Detection Method | Electrode | Linear Range | LOD | Recovery (RSD) | Assay Time | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| CEA | CEA | $MoS_2$ NS | TMB/$H_2O_2$ | Colorimetry | – | 50 – 1000 ng/mL | 50 ng/mL | 91.0–113.7% (3.4–11.0%) | – | [70] |
| | CEA | Pt NPs/Fe-MOF | oPD/$H_2O_2$ | DPV | GCE | 0.003–15.0 ng/mL | 1.0 pg/mL | 92.79–98.20% (< 6.0%) | – | [71] |

nanozyme-based single-mode aptamer biosensors for the detection of other protein tumor biomarkers, discuss their potential application in the detection of real human blood samples, and compare the detection performance of individual sensors in Table 4.

HER2-positive breast cancer is characterized by amplification of the HER2 gene, which is associated with more aggressive tumor growth, increased risk of metastasis, and poorer prognosis than other subtypes of breast cancer. Therefore, HER2 expression is a key tumor feature for diagnosis and treatment of breast cancer [72]. Ou et al. [73] constructed a sandwich-type electrochemical aptasensor to detect the breast cancer cellular biomarker HER2. The sensor consists of tetrahedral DNA nanostructures (TDNs)-nucleic acid aptamers as recognition probes, and flower-like nanozymes/horseradish peroxidase (HRP) as signaling nanoprobes. The principle of the developed biosensor is shown in Fig. 5A. First, a Pd@Pt-modified $Mn_3O_4$ flower-like nano-enzyme connected to aptamer 2 and the natural enzyme HRP constitutes the nanoprobe 1. Second, the TDNs-aptamer 1 is immobilized as a recognition element on the surface of a gold electrode (GE) via Au-S bonding for capturing the target HER2. Due to the large specific surface area, high stability, unique biocompatibility, and peroxidase mimetic activity of $Mn_3O_4$ and Pd@Pt floral nanoenzymes, nanoprobe 1 is able to amplify biosensor signals by catalyzing the oxidation of hydroquinone (HQ) by $H_2O_2$. after the binding of HER2 by TDNs-aptamer 1, an aptamer-HER2 protein-nanoprobe probe sandwich structure was formed on the GE surface. Finally, nanoprobe 2 consisting of complementary DNA (cDNA) of Pd@Pt/HRP/aptamer 2 was added to the sandwich structure to form dendritic DNA nanostructures, which significantly amplified the response signal intensity. The results showed that the sensor had a broad linear response in the range of 0.1 – 100.0 ng/mL, with a detection limit as low as 0.08 ng/mL. The designed method has great potential for constructing a variety of aptasensors for the effective and convenient detection of different biomarkers. However, the sensor not only needs to rely on the catalytic action of the natural enzyme HRP, but currently does not provide enough information for its application in clinical diagnosis.

Carbohydrate antigen 125 (CA125) is an O-glycosylated protein expressed on the surface of ovarian epithelial cells. This molecule is a widely used tumor-associated marker commonly used to diagnose ovarian cancer [74]. Tripathi et al. [75] used CA125 specific aptamer as recognition element and gold nanoparticles with peroxidase mimetic activity as a marker to detect CA125 in human serum by developing competitive aptamer-nanozyme lateral flow assay (ALFA). ALFA shows a low-intensity signal in the presence of CA125 and a high-intensity signal in the absence of the target. Gold nanoparticles captured on the test line catalyzed the oxidation of the DAB/$H_2O_2$ substrate and were analyzed for percent gray scale whiteness by acquiring images of standard strips before and after exposure to the DAB/$H_2O_2$ substrate. The linear detection range was 7.5 – 200 U/mL with a detection limit as low as 5.21 U/mL. The assay time for this test was only 20 min and the manufacturing cost was less than $1/strip. This work could provide a low-cost and stable diagnostic solution for people living in remote or isolated areas, especially in developing countries with limited medical facilities. Another study assembled CA125 aptamer and its c-DNA on the prepared $CeO_2$@MSF in sequence to construct the GP/CeO2@MSF/Apt/c-DNA colorimetric aptamer sensor (Fig. 5B) [76]. The sensor exhibited both excellent mimic oxidase and mimic phosphatase activities. In the presence of CA125, Apt specifically binds to the target CA125, and single-stranded c-DNA is released from the surface of GP/$CeO_2$@MSF/Apt, which is catalyzed for hydrolysis by exonuclease I. The resulting phosphate ions are able to inhibit the phosphatase-mimicking activity of $CeO_2$ nanozymes. In the linear range of 1.0 – 10.0 U/mL, the detection limit of the sensor for CA125 was as low as 0.43 mU/mL. Although the sensor has good stability and high accuracy, it can only be used for single-target detection, limiting its wider application.

During malignant transformation of hepatocytes, several oncogenic cell signaling molecules such as Golgi protein 73 (GP73) and Glypican-3 (GPC3) are expressed and secreted into the bloodstream [77]. These signaling molecules may not only be involved in the malignant transformation of hepatocytes, but may also be early diagnostic indicators of hepatocarcinogenesis or specific targeting molecules for hepatocellular carcinoma treatment. In 2022, Jintao Liang's group at Guilin University of Electronic Science and Technology constructed a colorimetric biosensor for GP73 with peroxidase-like activity based on reduced graphene oxide-carboxymethyl chitosan-Hemin/platinum@palladium nanoparticles (RGO-CMCS-Hemin/Pt@Pd NPs) as shown in Fig. 5C [78]. GP73 was detected by measuring the UV absorption peak at 652 nm. The concentration of GP73 was linearly correlated with the absorbance in the range of 10.0 – 110.0 ng/mL with a detection limit of 4.7 ng/mL. This colorimetric biosensor was successfully applied to detect GP73 in spiked human serum samples, demonstrating the great potential of a high-sensitivity GP73 colorimetric biosensor for clinical detection. In the same year, another study of this group for GP73 detection was to construct a dual-signal sandwich-type electrochemical sensor for GP73 determination based on heme-reduced graphene oxide-manganese oxide (H-rGO-$Mn_3O_4$) nanozymes [79]. Immobilization of amino-modified aptamer 1 (Apt1) on H-rGO-$Mn_3O_4$ nanozymes as a recognition

**Table 4** Single-mode aptamer biosensors based on nanozymes for other protein tumor biomarkers detection

| Cancer type | Biomarker | Aptamer | Nanozyme | Substrate | Detection Method | Electrode | Linear Range | LOD | Sensitivity | Recovery (RSD) | Assay Time | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| – | HER2 | HER2 | $Mn_3O_4$ Pd@Pt | $HQ/H_2O_2$ | DPV | GE | 0.1 – 100 ng/mL | 0.08 ng/mL | – | – | – | [73] |
| Ovarian Cancer | CA125 | Aptamer 2.26 Aptamer 2.43 | Au | $DAB//H_2O_2$ | ALFA | – | 7.5 – 200 U/mL | 5.21 U/mL | – | 86.5 – 114.8% | – | [75] |
| | | CA125 | $CeO_2$ | $TMB/H_2O_2$ | Colorimetry | – | 1.0 – 10.0 U/mL | 0.43 mU/mL | – | 95.4–104.0% (< 6.7%) | – | [76] |
| HCC | GP73 | GP73 | RGO-CMCS-Hemin/Pt@Pd | $TMB/H_2O_2$ | Colorimetry | – | 10.0–110.0 ng/mL | 4.7 ng/mL | – | 98.2–107.0% (1.90–5.44%) | 120 min | [78] |
| | | GP73 | H-rGO-$Mn_3O_4$ | $TMB/H_2O_2$ | SWV | SPE | 0.01–100.0 ng/mL | 0.0071 ng/mL | 2.441 μA/μM/$cm^2$ | 98.66–121.11% | – | [79] |
| | GPC3 | GPC3 | H-R-Pt@Pd | $TMB/H_2O_2$ | Colorimetry | – | 10–300 ng/mL | 5.06 ng/mL | – | 105.94–116.33% (0.93– 3.08%) | – | [80] |
| | | GPC3 | H-rGO-Pd | $AgNO_3$/ $H_2O_2$ | DPV | SPE | 0.01–10.0 μg/mL 10.0–100.0 μg/mL | 3.30 ng/mL | 1.535 $\mu A \mu M^{-1}$ $cm^{-2}$ | 103.78–106.52% (1.89–8.81%) | 30 min | [81] |
| – | $VEGF_{165}$ | $VEGF_{165}$ | $(MnCo)Fe_2O_4$ | $4\text{-CN}/H_2O_2$ | PEC | FTO | $1\times10^{-2}$–$1\times10^{4}$ pg/mL | 0.1 fg/mL | – | 100.9–101.7% (2.12–2.15%) | 60 min | [83] |

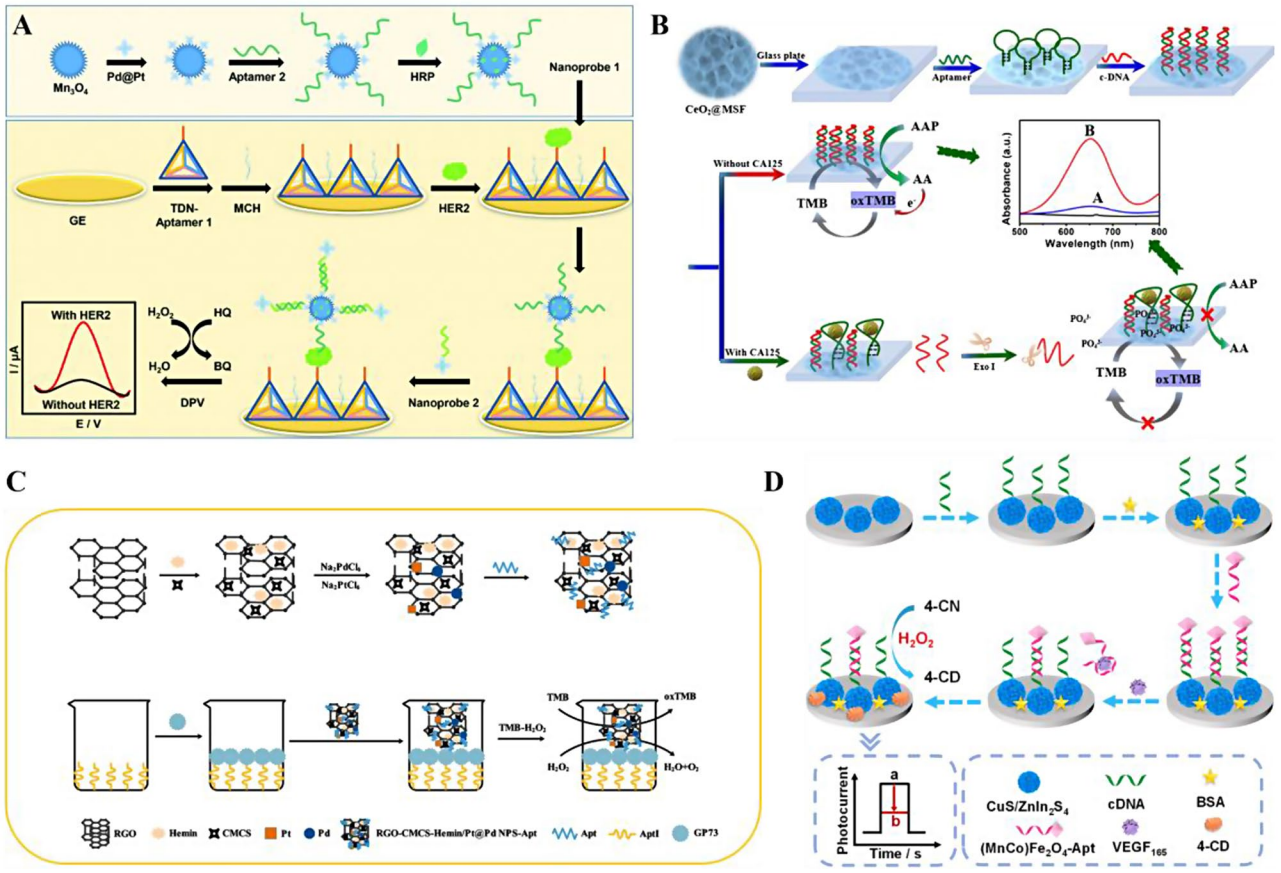


**Fig. 5** (**A**) Fabrication process of electrochemical dual aptamer biosensor for HER2 detection. Reprinted with permission from [73]. (**B**) Construction of CA125 colorimetric aptasensor. Reprinted with permission from [76]. (**C**) Schematic of the principle of the colorimetric biosensor based on RGO-CMCS-Hemin/Pt@Pd NPs for visual detection of GP73. Reprinted with permission from [78]. (**D**) Schematic diagram of the PEC aptasensor used to detect $VEGF_{165}$. Reprinted with permission from [83]

probe also serves as an in situ redox signaling indicator for the redox reaction of Hemin. Aptamer 2 (Apt2) was immobilized on the surface of screen-printed electrodes (SPEs) for the capture of GP73. Due to the excellent peroxidase activity of H-rGO-$Mn_3O_4$ nanozymes, they can catalyze the decomposition of $H_2O_2$ and the oxidation of substrate TMB to oxTMB. This sandwich-type electrochemical sensor has a detection limit of 0.0071 ng/mL in the range of 0.01 – 100.0 ng/mL. This dual-signal sandwich-type aptamer biosensor based on nanozymes could provide new insights for clinically effective diagnosis of hepatocellular carcinoma (HCC).

In 2023, the group initiated a study on the detection of GPC3 based on nanozymes. Researchers constructed a facile colorimetric aptasensor for GPC3 detection based on semi-reduced graphene oxide-Pt@Pd nanoparticles (H-rGO-Pt@Pd NPs) with peroxidase-like activity [80]. In this study, a GPC3 antibody was used as a capture probe, and a GPC3 aptamer modified on the surface of H-rGO-Pt@Pd NPs was used as a recognition probe. The H-rGO-Pt@Pd NPs were able to oxidize the colorless TMB to blue oxTMB in the presence of $H_2O_2$, which changed the absorbance of the system at 652 nm, and realized the sensitive colorimetric detection of GPC3. The linear range of the sensor was 10 – 300 ng/mL, and the detection limit was 5.06 ng/mL. In addition, the sensor can be used for the detection of GPC3 in human serum. The high selectivity, maneuverability and good sensitivity of this method indicate the potential of GPC3 detection in the field of clinical HCC diagnosis. Their other work constructed an ultrasensitive electrochemical biosensor for GPC3 detection based on heme-reduced graphene oxide-palladium nanoparticles (H-rGO-Pd NPs) nanozymes to enhance the signal amplification strategy of silver deposition [81]. This study also utilized the synergistic effect of GPC3 antibody and GPC3 aptamer on GPC3. H-rGO-Pd NPs with peroxidase-like properties induced the reduction of Ag ions in $H_2O_2$ and $AgNO_3$ solutions, which led to the deposition of Ag NPs on the surface of Au NPs@rGO/SPE. The response value of DPV was linearly correlated with the concentration of GPC3 in the range of 10.0–100.0 μg/mL, with a detection limit of 3.30 ng/mL. The electrochemical biosensor showed good recoveries for the detection of GPC3 levels

in real serum samples, confirming the applicability of the sensor in practical applications.

$VEGF_{165}$ is an important regulator of angiogenesis, and its altered expression level and structural variation play an important role in cancer development and are associated with overall survival and treatment response of cancer patients [82].

Yang et al. [83] constructed a photoelectrochemical (PEC) aptasensor for the detection of $VEGF_{165}$ based on a synthetic Cu/$ZnIn_2S_4$ flower-like heterojunction and (MnCo)$Fe_2O_4$ nanozymes. As shown in Fig. 5D, the prepared CuS/$ZnIn_2S_4$ is first fixed on the fluorine-doped tin oxide (FTO) electrode, which helps to generate a strong and stable photocurrent. Then, the cDNA of $VEGF_{165}$ aptamer and the (MnCo)$Fe_2O_4$-Apt modified $VEGF_{165}$ aptamer ((MnCo)$Fe_2O_4$-Apt) were gradually fixed, and the PEC signal showed a downward trend. Due to the excellent peroxisase-like activity of the (MnCo)$Fe_2O_4$ nanozymes, in the presence of $H_2O_2$, the (MnCo)$Fe_2O_4$ nanozyme catalyzes the oxidation of 4-chloro-1-naphthol (4-CN) in situ, forming an insoluble precipitate on the photoanode, resulting in sharp attenuation of the PEC signal. After introducing the target $VEGF_{165}$, the (MnCo)$Fe_2O_4$-modified $VEGF_{165}$ aptamer was released from the PEC aptamer sensing platform due to its high specificity and affinity to the target $VEGF_{165}$, terminating the color precipitation reaction and finally restoring the PEC signal. The developed PEC sensor has a wide linear range of $1\times10^{-2}$–$1\times10^{4}$ pg/mL with detection limits as low as 0.1 fg/mL. The study provides some valuable insights for building clinical tests for other ultra-sensitive cancer biomarkers.

## Dual-mode sensing strategies

Single-mode sensing strategies are relatively simple, low-cost, and more direct for data collection and analysis of a single signal, which can usually achieve faster response speeds. However, with the increasing application of nanozymes in biosensing, single-mode nanozyme aptamer sensors have low specificity in different detection environments, lack cross-validation mechanisms, are easily interfered by complex backgrounds, and have limited accuracy [84, 85]. This has led to growing interest among researchers to develop dual-mode nanozyme aptasensor, which integrates two distinct sensing mechanisms or modalities to enhance the detection and quantification of target molecules. Expectedly, dual-mode sensors will not only mitigate false positive signals associated with variations in detection conditions and operations, but also extend the detection range and improve the sensitivity.

Various types of nanozyme-based dual-mode sensors have been reported, such as colorimetric/fluorescence [86], electrochemical/colorimetric [87], colorimetric/photothermal [88], photoelectric chemistry/colorimetric [89]. Kim et al. [90] developed a colorimetric and electrochemical dual-mode sensing platform for the breast cancer biomarker thioredoxin 1 (TRX1) based on the excellent peroxidase mimetic and electrocatalytic properties of Prussian blue nanoparticles (PBNPs), achieving selective and sensitive detection of TRX1. Two completely independent signal paths in a dual-mode sensor effectively confirm the detection results, which further improves the sensitivity and accuracy of the dual-mode sensor. This section will introduce and discuss in detail the research progress of nanozyme-based dual-mode aptasensors, especially those with high sensitivity and strong anti-interference ability for the detection of tumor biomarkers such as CTCs, exosomes, and prostate-specific antigen (PSA). We will also discuss their potential applications in analyzing real human blood samples. A comparison of the detection performance of each sensor is provided in Table 5.

**Table 5** Dual-mode nanozyme-based aptamer biosensors for tumor biomarker detection

| Biomarker | Aptamer | Nanozyme | Substrate | Detection Method | Electrode | Linear Range | LOD | Recovery (RSD) | Assay Time | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| MCF-7 CTCs | SYL3C | MOF@Pt@MOF | TMB/$H_2O_2$ | SWV<br>Colorimetry | Au/rGO/ITO<br>– | 5 – $5\times10^5$ cells/mL | 5 cells/mL | 100.0 – 109.0% (0.6 – 2.3%) | – | [91] |
| MCF-7 exosomes | CD63 EpCAM | TMB-GQDzymes | TMB/$H_2O_2$ | Colorimetry<br>Photothermal analysis | –<br>– | 0 – $4\times10^4$ particles/μL<br>$2\times10^3$–$4\times10^4$ particles/μL | 1027 particles/μL<br>2170 particles/μL | – | – | [66] |
| | CD63 | $MoS_2$- Au@Pt | TMB/$H_2O_2$ | Chronoamperometry<br>Colorimetry | Au<br>– | 10 – $10^9$ particles/mL<br>$10^4$–$10^9$ particles/mL | 9.3 particles/mL<br>$4.2\times10^3$ particles/mL | > 93% (< 8%) | – | [92] |
| PSA | PSA | PtNP@$Co_3O_4$ | TMB/$H_2O_2$ | DPV<br>Colorimetry | SPE<br>– | 0.01 – 10 ng/mL<br>0.01 – 15 ng/mL | 0.0079 ng/mL<br>0.01 ng/mL | 97.03 – 105.75% | – | [93] |

### Detection of circulating tumor cells

Zhao et al. [91] proposed a dual-mode cellular sensing strategy based on a DNA walker and MOF@Pt@MOF nanozymes to capture and detect MCF-7 CTCs. Figure 6A shows the preparation process of MOF@Pt@MOF nanozyme-modified signal probe (SP). In this study, a SYL3C aptamer with high specificity for EpCAM protein was used to capture MCF-7 cells. In Fig. 6B, the SYL3C aptamer is prehybridized with its cDNA and modified on magnetic nanospheres (MNs). When MCF-7 CTCs are present, cDNA can be released through the specific recognition of CTCs and aptamers and separated by magnets, subsequently triggering the DNA walking process. In the process of DNA walking, the cDNA chain can trigger DNA replacement reaction through SP, releasing the cDNA chain to continue to participate in

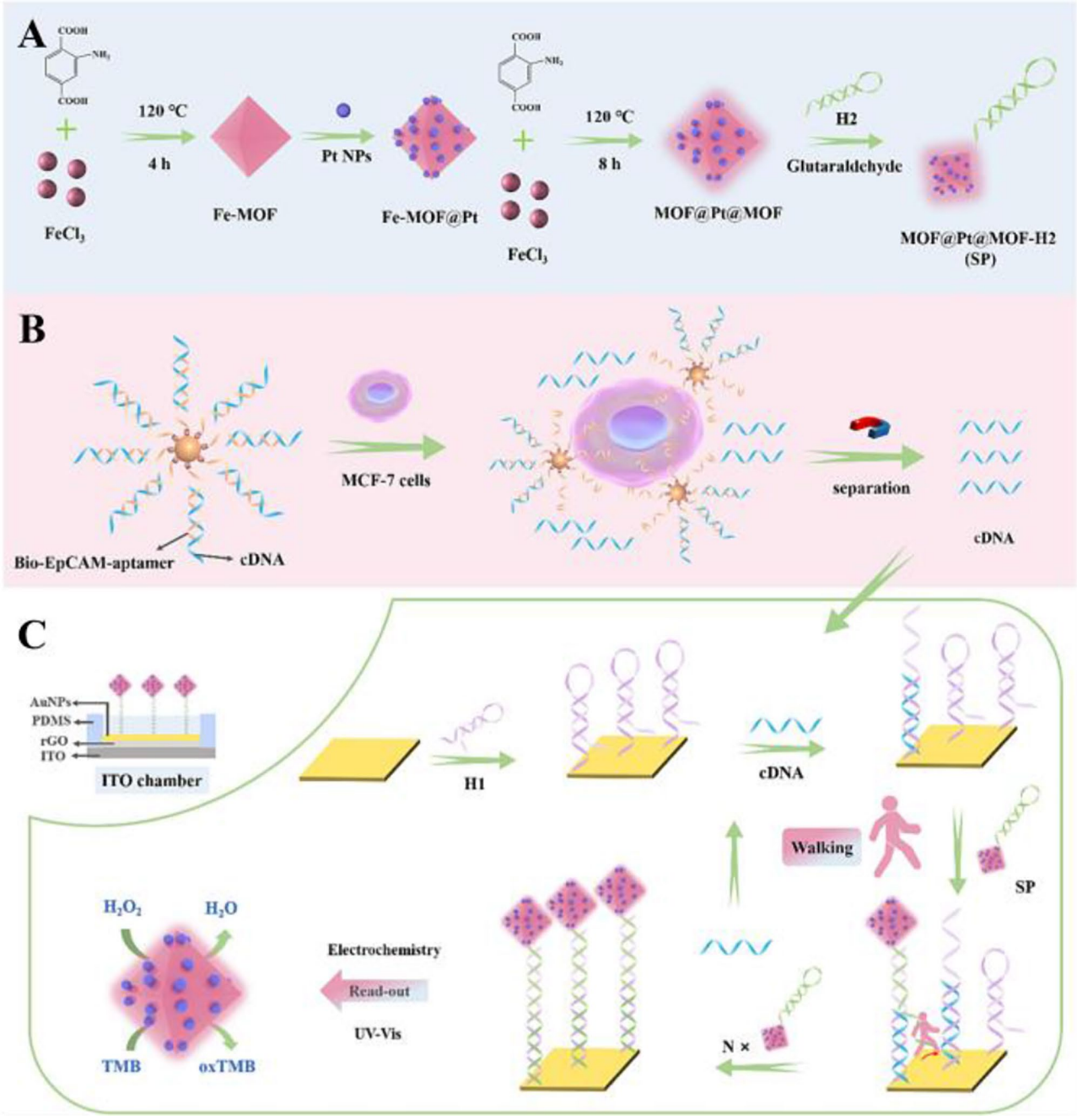


**Fig. 6** (**A**) Preparation process of MOF@Pt@MOF-H2. (**B**) Magnetic separation and release of cDNA chains in the presence of MCF-7 cells. (**C**) Planing map of ITO chamber and detection mechanism of CTCs sensor developed by DNA walkers and MOF-on-MOF nanozymes.
Reprinted with permission from [91].

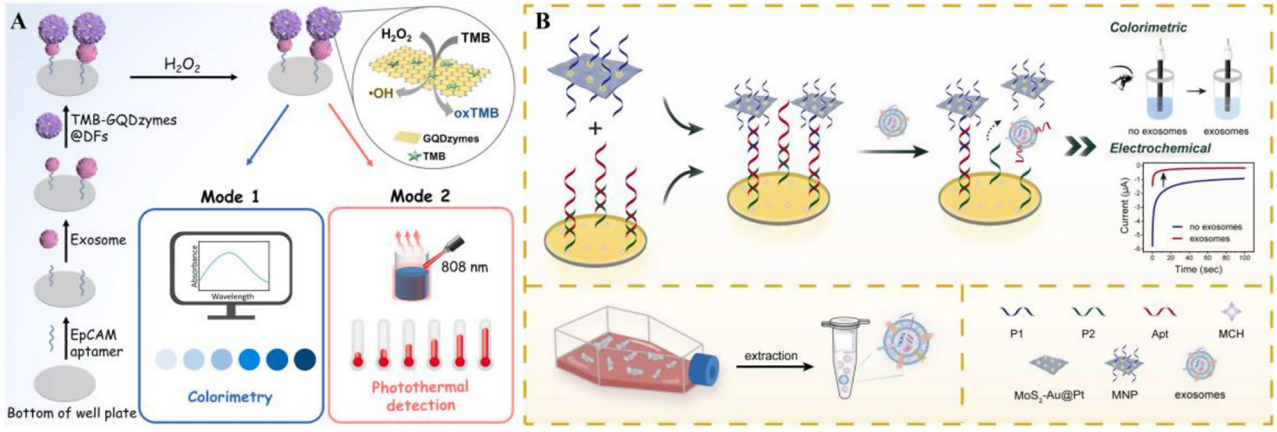


**Fig. 7** (**A**) Schematic diagram of the colorimetric/photothermal dual-mode biosensor for the detection of MCF-7 cell-derived exosomes based on dual aptamer recognition. Reprinted with permission from [66]. (**B**) The principle of electrochemical/colorimetric dual-mode sensitive detection sensor for exosomes based on $MoS_2$-Au@Pt nanozyme. Reprinted with permission from [92]

the cycle, thus amplifying the output signal. In addition, due to the good peroxidase activity of MOF@Pt@MOF nanozymes, dual-mode signal output by electrochemical and colorimetric method can be achieved by ITO chamber (Fig. 6C), so as to achieve the determination of CTCs. The DNA walker and MOF@Pt@MOF nanozyme have excellent synergistic signal amplification capabilities. The dual-mode cell sensor has a comprehensive linear range of $5-5\times10^5$ cells/mL and a detection limit as low as 5 cells/mL, and also performs well in human serum samples. In this study, the recognition process is carried out in the liquid phase, which improves the efficiency of the nucleic acid reaction, and the nanozyme probe is directly bound to the ITO chamber surface rather than the cell itself, reducing the stereoblocking effect.

### Detection of exosomes

Zhang et al. [66] proposed a sensitive and selective colorimetric/photothermal dual-mode method for the detection of MCF-7 cell exosomes by using TMB-loaded graphene quantum dot nanodopes (TMB-GQDzymes) encapsulated in DNA nanoflowers (DFs) as probes combined with the rolling circle amplification (RCA) self-assembly effect. As shown in Fig. 7A, to achieve specific detection, EpCAM aptamers for capturing MCF-7 cell exosomes were immobilized on the well plates, while CD63 aptamer sequences were modified in the DFs for DFs to be attached to the exosomes. The resulting TMB oxidation product oxTMB not only has enhanced characteristic absorption at 650 nm, but also produces a near-infrared laser-driven photothermal effect under 808 nm laser irradiation, thus realizing dual-mode detection of exosomes. The absorbance change ΔA was linearly related to exosome concentration in the range of $0-4\times10^4$ particles/μL, and the temperature increment ΔT was linearly related to exosome concentration in the range of $2\times10^3$–$4\times10^4$ particles/μL was linear with exosome concentration, and the detection limits were 1027 particles/μL (colorimetric assay) and 2170 particles/μL (photothermal assay), respectively. In addition, the sensing platform performed well in distinguishing breast cancer patients from healthy individuals in serum sample analysis. The dual-mode biosensor proposed in this study is expected to open up a promising future for exosome detection in biological research and clinical applications.

Combining the high catalytic activity of gold-platinum core-shell nanoparticle-modified molybdenum disulfide nanozymes ($MoS_2$-Au@Pt) and the specific recognition ability of Apts, Gong et al. [92] designed a two-in-one sensing platform for the detection of exosomes derived from the human breast cancer cell line MCF-7. As shown in Fig. 7B, probe P1 was immobilized on the surface of $MoS_2$-Au@Pt nanozymes via Au-S bonds to form a $MoS_2$-based signal amplified nanoprobes (MNP). Due to the peroxidase-like activity and excellent catalytic ability of $MoS_2$-Au@Pt, the MNP could effectively catalyze the oxidation of TMB in the presence of $H_2O_2$, resulting in enhanced electrochemical and colorimetric responses. In the presence of the target exosome, the preferential reaction between the CD63 aptamer and the exosome triggers the dissociation of the sandwich structure and the release of MNP from the electrode surface, resulting in a decrease in the electrochemical signal intensity and solution color. The electrochemical signal varied linearly with the exosome concentration in the range of $10-10^9$ particles/mL, and the absorbance had a linear relationship with the exosome concentration in the range of $10^4$–$10^9$ particles/mL, with detection limits of 9.3 particles/mL (electrochemical method) and $4.2\times10^3$ particles/mL (colorimetric method), respectively. This dual-mode sensing platform has high recoveries and low relative standard deviations for the detection of different concentrations

of MCF-7-derived exosomes in 5% human serum. This dual-mode sensing platform can mutually verify the assay results and minimize the errors caused by the assay conditions and operation, which opens up a new way for the accurate and sensitive detection of biomolecules.

#### Detection of PSA

Cao et al. [93] prepared a novel synergistic PtNP@$Co_3O_4$ hollow nanopolyhedron with peroxidase-like activity as a nanozyme and constructed a dual-mode sensor for PSA detection by combining electrochemical and colorimetric sensing strategies. When PSA is present, both the aptamer-modified magnetic beads and the PtNP@$Co_3O_4$ nanozyme bind specifically to PSA to form sandwich structure. The sandwich structure was separated from the PSA mixture using the magnetic effect of the magnetic beads, and the PtNP@$Co_3O_4$ nanozymes were able to catalyze the redox reaction between $H_2O_2$ and TMB, which produced quantitative electrochemical and colorimetric reactions simultaneously in homogeneous solution. The linear ranges of both the electrochemical channel (0.01 – 10 ng/mL) and the colorimetric channel (0.01 – 15 ng/mL) could meet the clinical demand for PSA detection (4 ng/mL), and the detection limits of the electrochemical and colorimetric channels without the use of natural enzyme were 0.0079 ng/ mL and 0.01 ng/ mL, respectively. This study provides a promising solution for the development of a simple, rapid, reliable, and ultrasensitive dual-channel homogeneous biosensor, which is expected to be a powerful tool for prostate cancer diagnosis.

In summary, dual-mode sensors exhibit enhanced detection capabilities with lower limits and an extended linear range. This study focuses on dual-mode aptamer biosensors utilizing nanozymes, which predominantly employ colorimetric sensing strategies. This is primarily due to the ability of nanozymes to catalyze the TMB/$H_2O_2$ reaction, generating oxTMB and resulting in significant color changes that are easily discernible to the naked eye. Furthermore, incorporating additional sensing methodologies—such as electrochemical and photothermal analyses—alongside colorimetric techniques can facilitate cross-validation of detection results, thereby enhancing the overall accuracy and reliability of the sensors.

## Conclusions

This review examines the latest advancements in nanozyme-based aptamer biosensors for tumor biomarker detection, focusing primarily on various sensing modalities including electrochemical, colorimetric, fluorescent, surface-enhanced Raman scattering (SERS), photothermal, and photoelectrochemical biosensors. The combination of aptamer biosensors with nanozymes has markedly elevated analytical performance, establishing them as essential tools for precision cancer diagnosis and treatment. Relevant studies are categorized into single-mode and dual-mode sensing strategies, emphasizing their applications for detecting crucial tumor biomarkers such as circulating tumor cells (CTCs), exosomes, and carcinoembryonic antigen (CEA). In these detection methodologies, nanozymes primarily function as signal probes. Their inherent catalytic properties facilitate signal amplification, and when paired with highly specific aptamer recognition probes, the overall detection efficacy of the sensors experiences substantial enhancement.

A comparative analysis of single-mode versus dual-mode sensors targeting the same biomarker reveals that dual-mode sensors can achieve lower detection limits and maintain a broader linear range. Additionally, the dual-channel detection capability of dual-mode sensors allows for cross-validation of results, thus ensuring greater accuracy and providing more comprehensive information about the sample.

Despite their superior performance, the development of dual-mode sensors necessitates careful design of biorecognition molecules that accommodate both detection modalities. Moreover, the fixation method of recognition elements must address the requirements of both detection strategies. Increased complexity comes with dual-mode sensing, as it is crucial to ensure that detection signals don't interfere with one another while maintaining the sensor's stability and repeatability. Current designs often overlook the detection time associated with dual-mode sensors, which could enhance their practicality in clinical applications.

As research progresses, future investigations should prioritize the following areas: (1) Development of Dual-Mode Sensor Strategies: Create streamlined approaches for synchronous detection in dual-mode sensors that simplify the overall detection process. (2) Simultaneous Biomarker Detection: Advance sensor technology to facilitate the simultaneous detection of multiple biomarkers, focusing on miniaturization and integration to enhance throughput. (3) Advanced Recognition Mechanisms: Explore dual-aptamer or multi-aptamer systems to increase the specificity and sensitivity of sensors. (4) Exploration of Nanozyme Properties: Investigate the multifunctional catalytic abilities of nanozymes and the synergistic effects of various nanozyme combinations to enhance signal transduction performance.

These innovations promise to significantly elevate sensor technology and broaden its applications across multiple fields. Nanozyme-based aptasensors exhibit considerable potential, and future advancements in transduction technologies, development of novel nanozymes and catalytic substrates, and the introduction of new bioreceptors will further propel tumor biomarker detection

technologies. This progress will enable biosensors to play a more significant role in precise clinical diagnostics and point-of-care applications.

**Abbreviations**

| | |
|---|---|
| CTCs | Circulating tumor cells |
| EVs | Extracellular vesicles |
| ELISA | Enzyme-linked immunosorbent assay |
| OXD | Oxidase |
| CAT | Catalase |
| SELEX | Systematic evolution of ligands by exponential enrichment |
| SWV | Square wave voltammetry |
| SERS | Surface enhanced Raman scattering |
| BC | Breast cancer |
| MUC-1 | Mucin 1 |
| GCEs | Glassy carbon electrodes |
| Apt | Aptamers |
| $VEGF_{165}$ | Vascular endothelial growth factor 165 |
| oxTMB | Oxidized TMB |
| h-JHNzyme | Hollow Janus hybrid nanozyme |
| 4-MPB | 4-mercaptophenylboronic acid |
| WB | Western blotting |
| oPD | o-phenylenediamine |
| LDA | Linear discriminant analysis |
| TDNs | Tetrahedral DNA nanostructures |
| GE | Gold electrode |
| cDNA | Complementary DNA |
| ALFA | Aptamer-nanozyme |
| ctDNA | Circulating tumor DNA |
| ctRNA | Circulating tumor RNA |
| Real-time PCR | Real-time polymerase chain reaction |
| POD | Peroxidase |
| SOD | Superoxide dismutase |
| CV | Cyclic voltammetry |
| DPV | Differential pulse voltammetry |
| RT-PCR | Reverse transcription polymerase chain reaction |
| rGO/AuNPs | Reduced graphene oxide/gold nanoparticle composites |
| MGCE | Magnetic glassy carbon electrode |
| EpCAMs | Epithelial-specific cell adhesion molecules |
| HFAuNSs | Highly fractalized gold nanostructures |
| TMB | 3,3′,5,5′-tetramethylbenzidine |
| ALL | Acute lymphoblastic leukemia |
| HCR | Hybridization chain reaction |
| MOFs | Metal-Organic Frames |
| HER2 | Human epidermal growth factor receptor 2 |
| DAP | 2,3-diaminophenazine |
| CEA | Carcinoembryonic antigen |
| HRP | Horseradish peroxidase |
| HQ | Hydroquinone |
| CA125 | Carbohydrate antigen 125 |
| GP73 | Golgi protein 73 lateral flow assay |
| GPC3 | Glypican-3 |
| HCC | Hepatocellular carcinoma |
| FTO | Fluorine-doped tin oxide |
| PSA | Prostate-specific antigen |
| MNs | Magnetic nanospheres |
| DFs | DNA nanoflowers |
| MNP | $MoS_2$-based signal amplified nanoprobes |
| AFB1 | Aflatoxin B1 |
| PBNPs | Prussian blue nanoparticles |
| SPEs | Screen-printed electrodes |
| PEC | Photoelectrochemical |
| 4-CN | 4-chloro-1-naphthol |
| SP | Signal probe |
| TMB-GQDzymes | TMB-loaded graphene quantum dot nanodopes |
| RCA | Rolling circle amplification |
| FDA | Food and drug administration |
| TRX1 | Thioredoxin 1 |

**Acknowledgements**
Not applicable.

**Author contributions**
KW: Literature review, drafting and revising the manuscript; XL, JYW and YL: manuscript review, and editing; XL, MX and JJW: editing, supervision, and administration. All authors read and approved the final manuscript.

**Funding**
This research was funded by Natural Science Foundation of Chongqing, China (NO. CSTB2022NSCQ-MSX0560), the National foreign expert project (NO. S20240042).

**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 29 November 2024 / Accepted: 29 January 2025
Published online: 07 February 2025

**References**

1. Chowdhury NA, Wang L, Gu L, Kaya M. Exploring the potential of sensing for breast Cancer detection. Appl Sci. 2023;13:9982.
2. Smit DJ, Pantel K. Circulating tumor cells as liquid biopsy markers in cancer patients. Mol Aspects Med. 2024;96:101258.
3. Pessoa LS, Heringer M, Ferrer VP. ctDNA as a Cancer Biomarker: a broad overview. Crit Rev Oncol Hematol. 2020;155:103109.
4. Wang X, Wang L, Lin H, Zhu Y, Huang D, Lai M, et al. Research progress of CTC, ctDNA, and EVs in cancer liquid biopsy. Front Oncol. 2024;14:1303335.
5. Kan C-M, Pei XM, Yeung MHY, Jin N, Ng SSM, Tsang HF, et al. Exploring the role of circulating cell-free RNA in the development of Colorectal Cancer. IJMS. 2023;24:11026.
6. Füzéry AK, Levin J, Chan MM, Chan DW. Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. Clin Proteom. 2013;10:13.
7. Díaz-Fernández A, Ferapontova EE. Electrochemical ELASA: improving early cancer detection and monitoring. Anal Bioanal Chem. 2023;415:3831–46.
8. Koizumi Y, Furuya D, Endo T, Asanuma K, Yanagihara N, Takahashi S. Quantification of Wilms' tumor 1 mRNA by digital polymerase chain reaction. Int J Hematol. 2018;107:230–4.
9. Yan B, Reinhard BM. Identification of Tumor cells through Spectroscopic profiling of the Cellular Surface Chemistry. J Phys Chem Lett. 2010;1:1595–8.
10. Lee JY, Lee S, Lee H, Tran TTP, Kim BC, Rhee WJ. In situ simultaneous detection of surface protein and microRNA in clustered Extracellular vesicles from Cancer Cell Lines using Flow Cytometry. ACS Biomater Sci Eng. 2023;9:6369–78.
11. Hsu C-C, Yang Y, Kannisto E, Zeng X, Yu G, Patnaik SK, et al. Simultaneous detection of Tumor Derived Exosomal Protein–MicroRNA pairs with an Exo-PROS Biosensor for Cancer diagnosis. ACS Nano. 2023;17:8108–22.
12. Chen S, Yu Z, Wang Y, Tang J, Zeng Y, Liu X, et al. Block-Polymer-restricted sub-nanometer pt Nanoclusters Nanozyme-enhanced immunoassay for monitoring of Cardiac Troponin I. Anal Chem. 2023;95:14494–501.
13. Li Z-F, Dong J-X, Vasylieva N, Cui Y-L, Wan D-B, Hua X-D, et al. Highly specific nanobody against herbicide 2,4-dichlorophenoxyacetic acid for monitoring of its contamination in environmental water. Sci Total Environ. 2021;753:141950.
14. Zhan L, Zhou JY, Li CM, Li YF, Huang CZ. Novel enzyme-linked immunoassay based on nanoceria as colorimetric substrate for foodborne pathogen detection. Microchem J. 2024;199:110028.

15. Shao Y, Zhou H, Wu Q, Xiong Y, Wang J, Ding Y. Recent advances in enzyme-enhanced immunosensors. Biotechnol Adv. 2021;53:107867.
16. Tao X, Wang X, Liu B, Liu J. Conjugation of antibodies and aptamers on nanozymes for developing biosensors. Biosens Bioelectron. 2020;168:112537.
17. Dadmehr M, Korouzhdehi B. Nanobiosensors for aflatoxin B1 detection, current research trends and future outlooks. Microchem J. 2023;194:109344.
18. Rahmanian H, Malekkiani M, Dadmehr M, Es'haghi Z, Moshirian-Farahi SS. A biosensor integrating the electrochemical and fluorescence strategies for detection of aflatoxin B1 based on a dual-functionalized platform. Anal Chim Acta. 2024;1323:343085.
19. Wei H, Wang E. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. Chem Soc Rev. 2013;42:6060.
20. Das B, Franco JL, Logan N, Balasubramanian P, Kim MI, Cao C. Nanozymes in Point-of-care diagnosis: an Emerging Futuristic Approach for Biosensing. Nano-Micro Lett. 2021;13:193.
21. Thamilselvan A, Kim MI. Recent advances on nanozyme-based electrochemical biosensors for cancer biomarker detection. TRAC Trends Anal Chem. 2024;177:117815.
22. Jiang X, Lv Z, Rao C, Chen X, Zhang Y, Lin F. Simple and highly sensitive electrochemical detection of Listeria monocytogenes based on aptamer-regulated pt nanoparticles/hollow carbon spheres nanozyme activity. Sens Actuators B. 2023;392:133991.
23. Li S, Wang F, Zhao B, Wang C, Wang Z, Wu Q. MnO2 nanoflowers based colorimetric and fluorescent dual-mode aptasensor for sensitive detection of aflatoxin B1 in milk. Anal Chim Acta. 2023;1279:341844.
24. Wang X, Zhang M, Pang X, Huang K, Yao Z, Mei X, et al. Comparative study of Pd@Pt nanozyme improved colorimetric N-ELISA for the paper-output portable detection of Staphylococcus aureus. Talanta. 2022;247:123503.
25. Li W, Fan G-C, Gao F, Cui Y, Wang W, Luo X. High-activity Fe3O4 nanozyme as signal amplifier: a simple, low-cost but efficient strategy for ultrasensitive photoelectrochemical immunoassay. Biosens Bioelectron. 2019;127:64–71.
26. Wu Y, Darland DC, Zhao JX. Nanozymes—Hitting Biosensing Target Sens. 2021;21:5201.
27. Dadmehr M, Korouzhdehi B, Hosseini M. Multimodal lab-on-a-tube detection of AFB1 based on fluorescence and nanozyme activity of DNA templated silver nanocluster: enhanced sensitivity through G-quadruplex formation. Microchem J. 2024;205:111127.
28. Xi J, Zhang R, Wang L, Xu W, Liang Q, Li J, et al. A nanozyme-based Artificial peroxisome ameliorates hyperuricemia and ischemic stroke. Adv Funct Mater. 2021;31:2007130.
29. Wang Y. Aptamer-based electrochemical analysis platform for tumor cells and biomarkers detection. J Electroanal Chem. 2024;960:118194.
30. Xu C, Tan Y, Zhang L-Y, Luo X-J, Wu J-F, Ma L, et al. The application of Aptamer and Research Progress in Liver Disease. Mol Biotechnol. 2024;66:1000–18.
31. Meng H-M, Fu T, Zhang X-B, Tan W. Cell-SELEX-based aptamer-conjugated nanomaterials for cancer diagnosis and therapy. Natl Sci Rev. 2015;2:71–84.
32. Jiang S, Liu M-S, Wang T, Zhang K-H. Evolution and Trends of aptamers in Cancer Research: a BibliometricAnalysis of the literature over the last decade. CMC. 2024;31.
33. Pei X, Liu J, Zhang Y, Huang Y, Li Z, Niu X, et al. Tetrahedral DNA-linked aptamer-antibody-based sandwich-type electrochemical sensor with Ag@Au core-shell nanoparticles as a signal amplifier for highly sensitive detection of α-fetoprotein. Microchim Acta. 2024;191:414.
34. Prakobkij A, Saenmuangchin R, Chunta S, Amatatongchai M, Citterio D, Jarujamrus P. Peroxidase-like activity of aptamer-gold nanoparticles for selective and sensitive fluorescence detection of low-density lipoproteins. ACS Appl Nano Mater. 2024;7:12356–65.
35. Davydova AS, Vorobyeva MA. Aptasensors based on non-enzymatic peroxidase mimics: current Progress and challenges. Biosensors. 2023;14:1.
36. Li C, Guo Z, Pu S, Zhou C, Cheng X, Zhao R, et al. Molybdenum Disulfide-Integrated Iron Organic Framework Hybrid Nanozyme-based Aptasensor for Colorimetric Detection of Exosomes. Biosensors. 2023;13:800.
37. Long J, Wang F, Zha G, Che K, Luo J, Deng Z. Colorimetric Aptasensor based on Fe3O4–Cu2 + nanozyme with intrinsic peroxidase-like activity in the detection of breast Cancer exosomes. j Biomed Nanotechnol. 2022;18:1084–96.
38. Jiang J, Xia J, Zang Y, Diao G. Electrochemistry/Photoelectrochemistry-Based immunosensing and aptasensing of Carcinoembryonic Antigen. Sensors. 2021;21:7742.
39. Donzella V, Crea F. Optical biosensors to analyze novel biomarkers in oncology. J Biophotonics. 2011;4:442–52.
40. Chen J, Chen Z, Meng C, Zhou J, Peng Y, Dai X, et al. CRISPR-powered opto-thermal nanotweezers: diverse bio-nanoparticle manipulation and single nucleotide identification. Light Sci Appl. 2023;12:273.
41. Feng Y-G, Zhu J-H, Wang X-Y, Wang A-J, Mei L-P, Yuan P-X, et al. New advances in accurate monitoring of breast cancer biomarkers by electrochemistry, electrochemiluminescence, and photoelectrochemistry. J Electroanal Chem. 2021;882:115010.
42. Gallerani G, Fici P, Fabbri F. Circulating Tumor cells: back to the future. Front Oncol. 2017;6.
43. Aoki M, Shoji H, Kashiro A, Takeuchi K, Shimizu Y, Honda K. Prospects for comprehensive analyses of circulating Tumor cells in Tumor Biology. Cancers. 2020;12:1135.
44. Shen C-C, Wu C-K, Chen Y-H, Wang J-X, Yang M-H, Zhang H. Advance in Novel methods for Enrichment and Precise Analysis of circulating Tumor cells. Chin J Anal Chem. 2021;49:483–95.
45. Cao R, Zhang M, Yu H, Qin J. Recent advances in isolation and detection of circulating tumor cells with a microfluidic system. CJCSP. 2022;40:213–23.
46. Lin H-I, Chang Y-C. Colorectal cancer detection by immunofluorescence images of circulating tumor cells. Ain Shams Eng J. 2021;12:2673–83.
47. Chen L, Zhou W, Ye Z, Zhong X, Zhou J, Chen S, et al. Predictive value of circulating Tumor cells based on Subtraction Enrichment for Recurrence Risk in Stage II Colorectal Cancer. ACS Appl Mater Interfaces. 2022;14:35389–99.
48. Sawai K, Goi T, Kimura Y, Koneri K. Presence of CD44v9-Expressing Cancer Stem cells in circulating Tumor cells and effects of Carcinoembryonic Antigen Levels on the prognosis of Colorectal Cancer. Cancers. 2024;16:1556.
49. Mei T, Lu X, Sun N, Li X, Chen J, Liang M, et al. Real-time quantitative PCR detection of circulating tumor cells using tag DNA mediated signal amplification strategy. J Pharm Biomed Anal. 2018;158:204–8.
50. Mușină A-M, Zlei M, Mentel M, Scripcariu D-V, Ștefan M, Aniței M-G, et al. Evaluation of circulating tumor cells in colorectal cancer using flow cytometry. J Int Med Res. 2021;49:030006052098021.
51. Tian L, Qi J, Qian K, Oderinde O, Liu Q, Yao C, et al. Copper (II) oxide nanozyme based electrochemical cytosensor for high sensitive detection of circulating tumor cells in breast cancer. J Electroanal Chem. 2018;812:1–9.
52. Tian L, Qi J, Qian K, Oderinde O, Cai Y, Yao C, et al. An ultrasensitive electrochemical cytosensor based on the magnetic field assisted binanozymes synergistic catalysis of Fe3O4 nanozyme and reduced graphene oxide/molybdenum disulfide nanozyme. Sens Actuators B. 2018;260:676–84.
53. Yang W, Fan L, Guo Z, Wu H, Chen J, Liu C, et al. Reversible capturing and voltammetric determination of circulating tumor cells using two-dimensional nanozyme based on PdMo decorated with gold nanoparticles and aptamer. Microchim Acta. 2021;188:319.
54. Yang L, Guo H, Hou T, Zhang J, Li F. Metal-mediated Fe3O4@polydopamine-aptamer capture nanoprobe coupling multifunctional MXene@Au@Pt nanozyme for direct and portable photothermal analysis of circulating breast cancer cells. Biosens Bioelectron. 2023;234:115346.
55. Su Y, Zhang Q, Miao X, Wen S, Yu S, Chu Y, et al. Spatially Engineered Janus Hybrid Nanozyme toward SERS Liquid Biopsy at Nano/Microscales. ACS Appl Mater Interfaces. 2019;11:41979–87.
56. Sun L, Li C, Yan Y, Yu Y, Zhao H, Zhou Z, et al. Engineering DNA/Fe–N–C single-atom nanozymes interface for colorimetric biosensing of cancer cells. Anal Chim Acta. 2021;1180:338856.
57. Liu J, Liu X, Yang L, Cai A, Zhou X, Zhou C, et al. A highly sensitive electrochemical cytosensor based on a triple signal amplification strategy using both nanozyme and DNAzyme. J Mater Chem B. 2022;10:700–6.
58. Chen Y, Li DJ, Wu TT, Ruan HB, Zheng MY. The mechanism of PDX in regulating cervical cancer HeLa cell proliferation and tumor formation. Eur Rev Med Pharmacol Sci. 2018;22(19):6268–73.
59. Zhu Y, Fang X, Lv X, Lu M, Xu H, Hu S, et al. A single aptamer-dependent sandwich-type Biosensor for the Colorimetric Detection of Cancer Cells via Direct Coordinately binding of Bare Bimetallic Metal–Organic Framework-based nanozymes. Biosensors. 2023;13:225.
60. Uyar R, Özçelikay-Akyıldız G, Kaya Sİ, Bereketoğlu Nergis S, Beşbinar Ö, Ünal MA, et al. Early cancer detection based on exosome biosensors in biological samples. Sens Actuators B. 2024;400:134886.
61. Mohammadi M, Zargartalebi H, Salahandish R, Aburashed R, Wey Yong K, Sanati-Nezhad A. Emerging technologies and commercial products in exosome-based cancer diagnosis and prognosis. Biosens Bioelectron. 2021;183:113176.
62. Sun Y, Haglund TA, Rogers AJ, Ghanim AF, Sethu P. Review: Microfluidics technologies for blood-based cancer liquid biopsies. Anal Chim Acta. 2018;1012:10–29.

63. Liu M-X, Zhang H, Zhang X-W, Chen S, Yu Y-L, Wang J-H. Nanozyme Sensor Array plus Solvent-Mediated Signal Amplification Strategy for Ultrasensitive Ratiometric Fluorescence Detection of Exosomal Proteins and Cancer Identification. Anal Chem. 2021;93:9002–10.
64. Kowal EJK, Ter-Ovanesyan D, Regev A, Church GM. Extracellular vesicle isolation and analysis by western blotting. In: Kuo WP, Jia S, editors. Extracellular vesicles. New York, NY: Springer New York; 2017. pp. 143–52.
65. Khodashenas S, Khalili S, Forouzandeh Moghadam M. A cell ELISA based method for exosome detection in diagnostic and therapeutic applications. Biotechnol Lett. 2019;41:523–31.
66. Zhang X, Zhu X, Li Y, Hai X, Bi S. A colorimetric and photothermal dual-mode biosensing platform based on nanozyme-functionalized flower-like DNA structures for tumor-derived exosome detection. Talanta. 2023;258:124456.
67. Song Z, Mao J, Barrero R, Wang P, Zhang F, Wang T. Development of a CD63 aptamer for efficient Cancer immunochemistry and immunoaffinity-based exosome isolation. Molecules. 2020;25:5585.
68. Zhao L, Cheng M, Liu G, Lu H, Gao Y, Yan X, et al. A fluorescent biosensor based on molybdenum disulfide nanosheets and protein aptamer for sensitive detection of carcinoembryonic antigen. Sens Actuators B. 2018;273:185–90.
69. Zhang K, Pei M, Cheng Y, Zhang Z, Niu C, Liu X, et al. A novel electrochemical aptamer biosensor based on tetrahedral DNA nanostructures and catalytic hairpin assembly for CEA detection. J Electroanal Chem. 2021;898:115635.
70. Zhao L, Wang J, Su D, Zhang Y, Lu H, Yan X, et al. The DNA controllable peroxidase mimetic activity of MoS 2 nanosheets for constructing a robust colorimetric biosensor. Nanoscale. 2020;12:19420–8.
71. Chen Q, He Q, Wang Y, Huang C, Lin Y, Wang J, et al. Aptamer-controlled peroxidase activity of platinum nanoparticles/Fe-MOF nanozyme for highly effective voltammetric detection of carcinoembryonic antigen. Microchem J. 2024;201:110609.
72. McGale J, Khurana S, Huang A, Roa T, Yeh R, Shirini D, et al. PET/CT and SPECT/CT imaging of HER2-Positive breast Cancer. JCM. 2023;12:4882.
73. Ou D, Sun D, Lin X, Liang Z, Zhong Y, Chen Z. A dual-aptamer-based biosensor for specific detection of breast cancer biomarker HER2 via flower-like nanozymes and DNA nanostructures. J Mater Chem B. 2019;7:3661–9.
74. Huo Q, Xu C, Shao Y, Yu Q, Huang L, Liu Y, et al. Free CA125 promotes ovarian cancer cell migration and tumor metastasis by binding Mesothelin to reduce DKK1 expression and activate the SGK3/FOXO3 pathway. Int J Biol Sci. 2021;17:574–88.
75. Tripathi P, Kumar A, Sachan M, Gupta S, Nara S. Aptamer-gold nanozyme based competitive lateral flow assay for rapid detection of CA125 in human serum. Biosens Bioelectron. 2020;165:112368.
76. Wen R, Wu X, Tian J, Lu J. A colorimetric aptasensor for CA125 determination based on dual catalytic performance of CeO2 nanozyme confined in macroporous silica foam. Microchim Acta. 2023;190:470.
77. Yao M, Fang R-F, Xie Q, Xu M, Sai W-L, Yao D-F. Early monitoring values of oncogenic signalling molecules for hepatocellular carcinoma. World J Gastrointest Oncol. 2024;16:2350–61.
78. Li X, Li S, Lv Q, Wang C, Liang J, Zhou Z, et al. Colorimetric biosensor for visual determination of Golgi protein 73 based on reduced graphene oxide-carboxymethyl chitosan-Hemin/platinum@palladium nanozyme with peroxidase-like activity. Microchim Acta. 2022;189:392.
79. Li G, Chen M, Wang B, Wang C, Wu G, Liang J, et al. Dual-signal sandwich-type aptasensor based on H-rGO-Mn3O4 nanozymes for ultrasensitive golgi protein 73 determination. Anal Chim Acta. 2022;1221:340102.
80. Li G, Li H, Wang C, Li X, Chen J, Liang J, et al. Colorimetric aptasensor for sensitive Glypican-3 detection based on Hemin-reduced oxide Graphene-Platinum@Palladium nanoparticles with peroxidase-like activity. IEEE Sens J. 2023;23:111–8.
81. Li G, Wang B, Li L, Li X, Yan R, Liang J, et al. H-rGO-Pd NPs Nanozyme Enhanced Silver Deposition Strategy for Electrochemical Detection of Glypican-3. Molecules. 2023;28:2271.
82. Al Kawas H, Saaid I, Jank P, Westhoff CC, Denkert C, Pross T, et al. How VEGF-A and its splice variants affect breast cancer development– clinical implications. Cell Oncol. 2022;45:227–39.
83. Yang H-Y, Wei J-J, Zheng J-Y, Ai Q-Y, Wang A-J, Feng J-J. Integration of CuS/ZnIn2S4 flower-like heterojunctions and (MnCo)Fe2O4 nanozyme for signal amplification and their application to ultrasensitive PEC aptasensing of cancer biomarker. Talanta. 2023;260:124631.
84. Liu Z, Chen R, Wang H, Wang C, Zhang X, Yang Y, et al. A colorimetric/electrochemical microfluidic biosensor using target-triggered DNA hydrogels for organophosphorus detection. Biosens Bioelectron. 2024;263:116558.
85. Zhang X, Zhi H, Zhu M, Wang F, Meng H, Feng L. Electrochemical/visual dual-readout aptasensor for Ochratoxin A detection integrated into a miniaturized paper-based analytical device. Biosens Bioelectron. 2021;180:113146.
86. Tan J, Geng W, Li J, Wang Z, Zhu S, Wang X. Colorimetric and fluorescence Dual-Mode biosensors based on peroxidase-like activity of the Co3O4 nanosheets. Front Chem. 2022;10:871013.
87. Wang G, Liu J, Dong H, Geng L, Sun J, Liu J, et al. A dual-mode biosensor featuring single-atom Fe nanozyme for multi-pesticide detection in vegetables. Food Chem. 2024;437:137882.
88. Lee HB, Son SE, Ha CH, Kim DH, Seong GH. Dual-mode colorimetric and photothermal aptasensor for detection of kanamycin using flocculent platinum nanoparticles. Biosens Bioelectron. 2024;249:116007.
89. Shen H, Yang H, Qileng A, Ma Y, Liang H, Meng J, et al. Programmable readout sensor for microRNA: CRISPR/Cas12a-assisted multi-amplification strategy activated photoelectrochemistry-colorimetry detection. Sens Actuators B. 2022;371:132585.
90. Kim JU, Kim JM, Thamilselvan A, Nam K-H, Kim MI. Colorimetric and Electrochemical Dual-Mode Detection of Thioredoxin 1 based on the efficient peroxidase-mimicking and Electrocatalytic Property of Prussian Blue Nanoparticles. Biosensors. 2024;14:185.
91. Zhao J, Long Y, He C, Leng Y, Huang Z, Ma Y, et al. Ultrasensitive detection of circulating tumor cells via DNA walker driven by a DNA circuit synergized with MOF-on-MOF nanozyme. Chem Eng J. 2024;479:147513.
92. Gong L, Chen B, Tong Y, Luo Y, Zhu D, Chao J, et al. Two-in-one sensing platform for the detection of exosomes based on molybdenum disulfide-based nanozymes. Sens Actuators B. 2024;398:134686.
93. Cao X, Liu M, Zhao M, Li J, Xia J, Zou T, et al. Synergetic PtNP@Co3O4 hollow nanopolyhedrals as peroxidase-like nanozymes for the dual-channel homogeneous biosensing of prostate-specific antigen. Anal Bioanal Chem. 2022;414:1921–32.


## Publisher's note