Progress in Nanomaterials-Based Optical and Electrochemical Methods for the Assays of Exosomes

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Abstract: Exosomes with diameters of 30–150 nm are small membrane-bound vesicles secreted by a variety of cells. They play an important role in many biological processes, such as tumor-related immune response and intercellular signal transduction. Exosomes have been considered as emerging and noninvasive biomarkers for cancer diagnosis. Recently, a large number of optical and electrochemical biosensors have been proposed for sensitive detection of exosomes. To meet the increasing demands for ultrasensitive detection, nanomaterials have been integrated with various techniques as powerful components. Because of their intrinsic merits of biological compatibility, excellent physicochemical features and unique catalytic ability, nanomaterials have significantly improved the analytical performances of exosome biosensors. In this review, we summarized the recent progress in nanomaterials-based biosensors for the detection of cancer-derived exosomes, including fluorescence, colorimetry, surface plasmon resonance spectroscopy, surface enhanced Raman scattering spectroscopy, electrochemistry, electrochemiluminescence and so on.

Keywords: exosomes, nanomaterials, circulating tumor biomarkers, electrochemical biosensor, optical biosensor

Introduction
Cancer is the main cause of death, and its occurrence and development is a gradual and complicated process. Early diagnosis and treatment of cancer can enormously improve the survival chance of cancer patients. Extracellular vesicles (EVs) are secreted by various cell types and circulate in different body fluids. They were first discovered as “cell junks” about 40 years ago.1,2 The lack of specific and reliable markers makes the clear assignation of EV subtypes extraordinarily difficult. According to operational terms for EV subtypes proposed by the International Society for Extracellular Vesicles (ISEV), EVs can be classified into three groups based on their size: exosomes, small EVs (sEVs) (< 200 nm), medium EVs (mEVs), and large EVs (lEVs).3 Exosomes are nanosized extracellular vesicles (EVs) (30–200 nm). For the convenience of the reader, this review used a relatively broad term of “exosomes” to refer to a heterogeneous mixture of sEVs less than 200 nm in size because it is increasingly utilized in bioassays.4 Compared to circulating tumor cells and circulating DNA, exosomes exist in body fluids (such as serum, urine and ascites) with higher abundance and stability.5 Emerging evidences suggest that most types of cancer secrete large numbers of exosomes that carry abundant molecular information stemming from parent tumor cells, including nucleic acids, proteins, bioactive lipids and metabolites.6,7
Exosomes can act as cellular messengers to deliver information between cells via endocytosis.\textsuperscript{8,9} They also play a key role in cancer metastasis and progression in the tumor microenvironment.\textsuperscript{10,11} Moreover, various unique cargos in exosomes represent meaningful physiological and pathological states of diseases.\textsuperscript{12,13} Therefore, exosomes have been recognized as the most reliable non-invasive biomarkers for the early diagnosis and cure of cancers.\textsuperscript{14–17}

Various exosomes-containing complex biological fluids have a plenty of nonvesicular macromolecules, such as proteins, proteases, nuclease and RNA complexes, which may interfere with the analysis of exosomes. For example, proteases and nuclease can digest the biorecognition elements such as antibodies and aptamers. Thus, the growing interests in exosomes and their potential applications in cancer detection have pushed researchers to develop various isolation and detection techniques. Normally, conventional isolation techniques, including ultracentrifugation, density gradient centrifugation and ultrafiltration, are mainly based on the physical properties of exosomes (eg size and density).\textsuperscript{5,18} However, these techniques confront of some problems, such as low purity, tedious procedures and requirement of expensive instruments. Traditional detection techniques utilized to quantify the isolated exosomes include nanoparticle tracking analysis (NTA), flow cytometry, Western blot, dynamic light scattering, scanning electron microscope (SEM), and transmission electron microscope (TEM).\textsuperscript{2,19–22} Although these methods have been popularly used, the problems of low reproducibility, time consuming, large sample demand and low sensitivity limit their further applications.

Over the past years, many types of proteins, such as tetraspanins (eg CD9, CD63, CD81 and CD82), adhesion molecules (eg integrins and lactadherin) and lipid rafts (eg cholesterol, phosphatidylserine and ceramide), have been found on the membrane of all types of exosomes.\textsuperscript{23,24} These biomolecules can be utilized as the targets for the detection of total exosomes. However, exosomes derived from various tumor cells exhibit different cancers-associated antigens on the surface. These distinct antigens have been used as the biomarkers for the determination of certain cancer-derived exosomes.\textsuperscript{25} For example, MCF-7-secreted exosomes with highly expressed MUC1 on the surface.\textsuperscript{26} Protein tyrosine kinase 7 and CD147 are over-expressed on the surface of human leukemic lymphoblasts (CCRF-CEM) and colorectal cancer cellular exosomes, respectively.\textsuperscript{27,28} These proteins are the promising markers for the isolation and detection of exosomes with the aid of biorecognition elements.\textsuperscript{29,30} By modifying magnetic beads (MBs), chips or microfluidics with antibodies toward exosome membrane markers, immunoaffinity-capture-based techniques are proposed for the isolation and enrichment of exosomes with high selectivity and specificity as well as intact biological activity.\textsuperscript{23,31–44} However, the expensive cost and instability of antibodies limit their practical applications. Aptamers and peptides are screened to act as biorecognition elements for the isolation and detection of exosomes by the virtue of low cost, small size and relatively excellent stability.\textsuperscript{45–47} Besides, exosomal glycans provide a valuable route to label exosomes through the interaction of lectin and glycan.\textsuperscript{48,49} Lipophilic cholesterol anchors can penetrate into the lipid bilayers for membrane modification, which have been regarded as the promising candidates for labeling of exosomes.\textsuperscript{50,51} In contrast to physical property-based isolation techniques, affinity-based isolation techniques exhibit much higher enrichment efficiency, facilitating the sensitive detection of exosomes.

In recent years, numerous novel biosensors with high sensitivity and efficiency have been established for the determination of exosomes by specific recognition, including colorimetry, fluorescence, surface plasmon resonance (SPR), surface enhanced Raman scattering (SERS) spectroscopy, mass spectrometry and electrochemical, electrochemiluminescent (ECL) and photoelectrochemical (PEC) assays.\textsuperscript{4,52–59} Moreover, many works based on microfluidic devices implemented with optical or electrochemical techniques have been reported for the comprehensive assays of exosomes.\textsuperscript{60–64}

With the growing development of nanotechnology, myriad nanomaterials and nanostructures have made a great impact on biosensing. Significant advances have made it possible to controllably prepare nanomaterials with various chemical composition, morphology and physicochemical characteristics. For example, nanomaterials with wonderful luminescence properties have been an important alternative to traditional dyes in optical assays, because of their excellent merits of adjustable emission wavelength, high luminescence quantum yield and good photostability. Thanks to the interesting size and shape-dependent localized surface plasmon resonance phenomenon, noble metal nanoparticles (NPs), especially gold and silver, have been widely used to enhance the signal intensity in SPR and SERS assays. Carbon-based nanomaterials (eg, carbon nanotubes and graphene oxide) with a high
surface-to-volume ratio and high electrical conductivity are always employed for electrode modification to accelerate the electron transfer and increase the electrode surface area. Moreover, recently, the photothermal and enzyme-mimic properties of nanomaterials have gained considerable interest toward the development of portable bioassays. Various nanomaterials-based signal amplification strategies, coupled with different detection techniques, have been developed for the ultrasensitive detection of biomolecules, including DNA/RNA, proteins, exosomes and cells. In exosomes detection, there are two mainly objectives, including improving the capture of exosomes and enhancing the performance of detection methods. For the former, magnetic beads (MBs) as the classical materials have been increasingly used to selectively capture exosomes from clinical samples, when being decorated with antibodies or aptamers.

In this review, the recent progress in nanomaterials-based biosensors for exosome detection was comprehensively summarized. The detection techniques mainly cover optical and electrochemical assays (Tables 1 and 2). Nanomaterials are involved in semiconductor quantum dots (QDs), metal NPs, metal oxides and sulfides, and carbon-based nanomaterials. The intent of this review is to impart insights into the versatile roles of nanomaterials in assays, and illustrate their potential benefits in further applications.

**Nanomaterials-Based Optical Biosensors for Exosome Detection**

**Fluorescence Biosensors**

Fluorescence biosensors have great advantages of simple operation, comparable sensitivity, and multiplex target detection capability. Exosome can be labeled with dyes or dye-modified biorecognition elements through various targeting strategies and then determined by fluorescence imaging or spectroscopy. However, the low fluorescence intensities of dyes always limits the sensitivity for exosome detection. Thus, several signal amplification strategies have been proposed to improve the detection sensitivity. For example, β-galactosidase-labeled antibody was used to label the captured exosomes in which β-galactosidase acted as the reporter enzyme to catalyze the decomposition of fluorescein-di-β-D-galactopyranoside, generating a strong fluorescence signal. Combined with nano-interface-based microfluidic platforms, exosomes were effectively enriched and sensitively detected.

For homogeneous fluorescence detection, it is one of the most powerful strategies to convert the detection of exosomes into the detection of DNA or others, generally producing a “one-to-many” amplification effect. Owing to the flexible structure, the corresponding DNA can be ultrasensitively and homogeneously detected by previously reported DNA-based signal amplification methods, such as terminal deoxynucleotidyl transferase (TdT)-mediated polymerization and hybridization chain reaction (HCR). For example, Gao et al reported a dual signal amplification method for indirect detection of exosomes based on the catalytic hairpin DNA cascade reaction (HDCR) and the self-assembly of DNA dimer on the surface of gold nanoparticles (Au NPs). In this work, streptavidin (SA)-modified MBs were labeled with biotin-modified CD63 aptamer and then bound with probe S through the hybridization. After the addition of exosomes, aptamers bound with CD63 on exosomes and the probe S was released to trigger the HDCR on the nanoparticle surface. Then, the opened metastable hairpin (HP) DNA probes captured the fluorescently-labeled DNA dendrimers. After several rounds of Y-shaped DNA assembly, the complexes of AuNPs, HP and DNA dendrimers were separated. With the aid of β-mercaptoethanol, the fluorescently-labeled dendrimers were released and the fluorescence signal was recorded for the determination of exosomes. This method showed an increased signal-to-noise ratio and had a linear detection range of $1.75 \times 10^5$ – $7.0 \times 10^6$ particles/μL. Pan et al reported a steric hindrance-controlled signal-amplified fluorescent strategy for exosome detection. In the absence of exosome, cholesterol-conjugated DNA 1 could hybridize with SA-modified DNA 2 into dsDNA with blunt ends. The formed dsDNA could not be recognized and extended by TdT enzyme because of the absence of single-stranded initiator (more than three deoxynucleotide residues). However, when exosomes were added, DNA 1 was inserted into the lipid membrane through the hydrophobic interaction between cholesterol and lipid bilayer. The huge steric hindrance of exosomes strongly inhibited the hybridization of DNA 1 and SA-modified DNA 2. Therefore, SA-modified DNA 2 could be extended by TdT enzyme to generate abundant G-quadruplex structures, increasing the fluorescence intensity. The “signal-on” method maintained high sensitivity and excellent selectivity for the assay of complex samples. Li et al developed a reversible nanoparticle for fluorescent detection of urinary exosomes by using a superparamagnetic conjunction and molecular beacon. As shown in Figure 1, prostate specific membrane antigen (PSMA) aptamer was modified on the
| Methods      | Type of Nanomaterials                        | Functions                                                                 | Cancer Types | Target Biomarkers | Response Time (min) | Detection Limit (Particles/μL) | Linear Range (Particles/μL) | Ref. |
|--------------|---------------------------------------------|---------------------------------------------------------------------------|--------------|-------------------|---------------------|-------------------------------|----------------------------|------|
| Fluorescence| Biorecognition elements-modified MNPs       | Capturing exosomes and transforming the detection of exosomes into the detection of DNA with DNA-based signal amplification | Liver cancer | CD63              | Not reported        | 0.1                           | 1–1 × 10^4                  | [83] |
|              |                                             |                                                                           | Not reported | CD63 and lipid bilayer | 160                   | 1.29 × 10^3               | 5.5 × 10^5–1.1 × 10^7      | [84] |
|              |                                             |                                                                           | Lung cancer  | CD63              | 65                   | 1 × 10^3                  | 1 × 10^2–1 × 10^6            | [85] |
|              |                                             |                                                                           | Lung cancer  | CD63              | 95                   | 0.1                        | 0.3–1 × 10^4                  | [86] |
|              |                                             |                                                                           | Liver cancer | CD63              | 255                   | 1.16 × 10^3               | 1.75 × 10^2–7 × 10^6        | [90] |
|              |                                             |                                                                           | Cervical cancer | CD63 and lipid bilayer | 245                   | 480                        | 1.66 × 10^2–1.66 × 10^6      | [91] |
|              |                                             |                                                                           | Prostate cancer | PSMA              | 210                   | 1 × 10^2                 | 1 × 10^2–1 × 10^5            | [92] |
| QDs          | Fluorescence labels                         |                                                                           | Lung cancer  | EpCam             | 60                   | 0.013                      | 5 × 10^2–5 × 10^5            | [94] |
|              |                                             |                                                                           | Oral squamous Cell carcinoma | CD63              | 30                   | 5 × 10^2                  | 5.0 × 10^2–1.0 × 10^4        | [95] |
|              |                                             |                                                                           | Pancreatic cancer | GPC-1             | 90                   | Not reported              | 1.0 × 10^3–5.0 × 10^6        | [96] |
|              |                                             |                                                                           | Human tongue squamous cell carcinoma | Lipid bilayer | 65                   | 2 × 10^3                  | 4 × 10^2–2 × 10^5            | [97] |
| CuNPs        |                                             |                                                                           | Liver cancer  | CD63 and lipid bilayer | 120                   | 4.8 × 10^4               | 7.5 × 10^3–1.5 × 10^7        | [98] |
| AuNPs        | Nanoquenchers                               |                                                                           | Leukemia cell | CD63 and nucleolin | 180                   | 1 × 10^2                  | 1 × 10^5–1 × 10^5            | [88] |
| GO           |                                             |                                                                           | Breast cancer | PSMA              | 40                   | 1.6 × 10^2               | 1.6 × 10^2–1.6 × 10^5        | [101]|
|              |                                             |                                                                           | Colorectal cancer | CD63 and EpCAM     | 40                   | 2.1 × 10^3               | 3 × 10^6–6 × 10^8            | [102]|
|              |                                             |                                                                           | Breast cancer  | GPC-1             | 128                   | 6.56 × 10^4               | 7.8 × 10^3–3.9 × 10^6        | [103]|
| M_2S_2-MWCNTs|                                             |                                                                           | Breast cancer  | CD63              | 70                   | 1.48 × 10^3               | 0–1.11 × 10^4               | [104]|
| Ti_3C_2 MXenes| Nanoquenchers and luminescent emitters     |                                                                           | Breast cancer  | CD63              | 70                   | 1.4                        | 10–1 × 10^6                 | [105]|
| AuNRs        | UCNPs                                      | Quenching the luminescence of UCNPs by AuNRs                              | Breast cancer  | CD63              | 250                   | 8.2                        | 20–2.0 × 10^4               | [99] |
| Abbreviations                      | Tissue                      | CD63, CD9, CD81, LMP1 and EGFR | CD63, CD9, GPC-1, EpCAM, and CD44V6 | CD9 and lipid bilayer | PSMA, Her2 and AFP | Pancreatic cancer                     | Electromagnetic hot spots to enhance Raman signals | AuNPs | Nanocarriers for enzymes | colorectal cancer | A33 and EpCAM | 220 | 6.7 × 10<sup>3</sup> | 9.75 × 10<sup>3</sup>–1.95 × 10<sup>6</sup> | [114] | AuNPs | Color indicators | Nasopharyngeal carcinoma | CD63 | 0.1–1 × 10<sup>3</sup> | [122] | AuNRs | Breast cancer | CD63 and lipid bilayer | Not reported | 1.6 × 10<sup>5</sup> | 1.4 × 10<sup>5</sup>–2.8×10<sup>5</sup> | [127] | AuNBP@MnO<sub>2</sub> NSs | Breast cancer | CD63 | 155 | 1.35 × 10<sup>2</sup> | 8.5 × 10<sup>5</sup>–8.5 × 10<sup>6</sup> | [128] | C-IONPs | Nanocarriers for enzymes | Ovarian cancer | CD63 | 1.25 × 10<sup>3</sup> | 6.25 × 10<sup>5</sup>–1.0 × 10<sup>6</sup> | [129] | AuNPs | Peroxidase-mimetic enzyme | Breast cancer | CD63 | 30 | 1.352 × 10<sup>6</sup> | 1.9 × 10<sup>5</sup>–3.38 × 10<sup>7</sup> | [130] | SWCNTs | Breast cancer | CD63 | 40 | 5.2 × 10<sup>5</sup> | 1.84 × 10<sup>6</sup>–2.21 × 10<sup>7</sup> | [131] | CuCo<sub>2</sub>O<sub>4</sub> nanorods | Breast cancer | CD63 | 160 | 4.5 × 10<sup>5</sup> | 5.6 × 10<sup>5</sup>–8.9 × 10<sup>6</sup> | [132] | SPR | Chip substrate | Lung cancer | PD-L1 | 50 | 0.02 | 10–1.0 × 10<sup>5</sup> | [145] | MNPs | Nanocarriers for enzymes | Lung cancer | CD63, EGFR and EpCAM | 55 | 2.37 × 10<sup>4</sup> | 3.125 × 10<sup>3</sup>–100 × 10<sup>4</sup> | [146] | AuNPs | Breast cancer | CD63 | 155 | 5 | Not reported | Not reported | [151] | SERS | Raman reporters with LSPR | Pancreatic cancer | CD63, GPC-1, EpCAM, and CD44V6 | 150 | Not reported | Not reported | [160] | Polydopamine-encapsulated Au@Ag NPs | Pancreatic cancer | CD63, GPC-1, EGFR, EpCAM, and MIF | 150 | Not reported | 0.544–272 | [161] | AuNS@4-MBA@Au | Hepatocellular Carcinoma | CD9 and lipid bilayer | 50 | 27 | 40–4.0 × 10<sup>7</sup> | [162] | Gold-silver-silver core-shell nanotrepangs | Hepatocellular Carcinoma | PSMA, Her2 and AFP | 120 | 35 | 1–1 × 10<sup>7</sup> | [163] | TP-DNA@AuNPs | Electromagnetic hot spots to enhance Raman signals | Breast cancer | EpCAM | 150 | 110 | 1 × 10<sup>3</sup>–1 × 10<sup>7</sup> | [164] | Polydopamine-encapsulated Au@Ag NPs | Polydopamine-functionalized iron oxide nanoparticles | CD63, GPC-1, EGFR, EpCAM, and MIF | 150 | Not reported | 0.544–272 | [161] | AuNS@4-MBA@Au | Polydopamine-functionalized gold nanoparticle | CD63, GPC-1, EpCAM, and CD44V6 | 150 | Not reported | Not reported | [164] | Polydopamine-encapsulated Au@Ag NPs | Polydopamine-functionalized gold nanoparticle | CD63, GPC-1, EpCAM, and CD44V6 | 150 | Not reported | 0.544–272 | [161] | Abbreviations: QDs, quantum dots; CuNPs, copper nanoparticles; AuNPs, gold nanoparticles; GO, graphene oxide; MWCNTs, multiwall carbon nanotubes; AuNRs, gold nanorods; UCNPs, upconversion nanoparticles; AuNBP@MnO<sub>2</sub> NSs, gold nanobipyramid@MnO<sub>2</sub> nanosheet nanostructures; C-IONPs, carboxyl group-functionalized iron oxide nanoparticles; SWCNTs, single-walled carbon nanotubes; Au@PDA NPs, polydopamine-functionalized gold nanoparticle; TP-DNA, triangular pyramid DNA; AuNS@4-MBA@Au, gold nanostar@4-mercaptobenzoic acid@nanoshell structures; PSMA, prostate-specific membrane antigen; GPC-1, glypican-1; EpCAM, epithelial cell adhesion molecule; LMP1, latent membrane protein 1; EGFR, epidermal growth factor receptor; PD-L1, programmed death ligand-1; CD44V6, CD44 variant isoform 6; MIF, migration inhibitory factor. |
| Methods                        | Type of Nanomaterials | Functions                                                                 | Cancer Types | Target Biomarkers | Response Time (min) | Detection Limit (Particles/μL) | Linear Range (Particles/μL) | Ref. |
|-------------------------------|-----------------------|---------------------------------------------------------------------------|--------------|-------------------|---------------------|------------------------------|----------------------------|------|
| Electrochemistry (direct detection) | AuNPs                | Improving the large surface area, accelerating electrical conductivity and facilitating the immobilization of biorecognition elements | Breast cancer | CD63              | 180                 | $4.82 \times 10^2$          | $5 \times 10^2$–$5 \times 10^3$ | [181] |
|                               | BPNSs and MOFs        |                                                                           | Breast cancer | CD63              | Not reported        | $1 \times 10^5$              | $1.3 \times 10^5$–$2.6 \times 10^5$ | [182] |
| Electrochemistry (sandwich detection) | AuNPs                | Nanocarriers for enzymes, electroactive molecules                         | Liver cancer  | CD63              | 255                 | 16.6                        | $21.6$–$7.5 \times 10^4$             | [189] |
|                               | Zr-MOF               |                                                                           | Glioblastoma  | EGFR, EGFRvIII, and phosphate groups outside of exosomes | 150                 | $7.83 \times 10^3$          | $9.5 \times 10^3$–$1.9 \times 10^7$ | [190] |
|                               | Zr-MOF and ZIF-8     |                                                                           | Breast cancer  | CD63 and phosphate groups outside of exosomes             | 190                 | 0.3                          | $1$–$1 \times 10^5$               | [191] |
|                               | ZIF-8                |                                                                           | Breast cancer  | CD63 and PD-L1   | 245                 | 0.334                       | $1$–$1 \times 10^5$               | [192] |
|                               | COFs@Au NPs          |                                                                           | Colorectal cancer | CD63 and amino acid residues outside of exosomes         | 170                 | $1.6 \times 10^4$          | $5 \times 10^3$–$5 \times 10^7$   | [193] |
|                               | Au-NPFe$_2$O$_3$NC    | Nanozymes to catalyze the redox reaction                                  | Placental choriocarcinoma | CD63 and PLAP | 90                  | 1                           | $1$–$1 \times 10^6$               | [194] |
|                               | Ti$_3$C$_2$ MXene@PB  | Showing electroactive properties or consisting of electroactive ions       | Ovarian cancer  | CD63              | 120                 | $2.29 \times 10^2$          | $5 \times 10^2$–$5.0 \times 10^5$ | [195] |
|                               | CdSe QDs             |                                                                           | Colorectal adenocarcinoma | CD63, HER-2 and FAM134B | 130                | $1 \times 10^3$          | $1 \times 10^2$–$1 \times 10^2$       | [196] |
| Electrochemiluminescence | Ru(II) markers-loaded SiNPs | Nanomaterials acts as nanocarriers for molecules | Hepatocellular carcinoma | CD63 | 60 | \(2 \times 10^2\)–7.5 \(\times 10^4\) | [212] |
|--------------------------|-----------------------------|-----------------------------------------------|--------------------------|------|----|-----------------|------|
| CDSe:Eu QDs              | Electrochemiluminescence emitter | Breast cancer | CD63 | 240 | 74.1 | 3.4 \(\times 10^2\)–1.7 \(\times 10^4\) | [210] |
| CdS QDs@CaCO\(_3\)       |                             | Breast cancer | EpCAM | 180 | 21 | 50–1 \(\times 10^5\) | [212] |
| g-C\(_3\)N\(_4\)@Galinstan-PDA |                 | Cervical cancer | GPC-1 | 120 | 31 | 50–1 \(\times 10^5\) | [211] |
| BPQDs@ Ti\(_3\)C\(_2\) MXene | Coreactants | Breast cancer | CD63 and EpCAM | 100 | 37 | 1.1 \(\times 10^2\)–1.1 \(\times 10^7\) | [214] |
| AuNPs@ Ti\(_3\)C\(_2\) MXene | Nanocatalysts | Cervical cancer | CD63 | 240 | 30 | 1 \(\times 10^2\)–1 \(\times 10^5\) | [215] |
| Ti\(_3\)C\(_2\) MXene    |                              | Breast cancer | EpCAM | 240 | 1.25 \(\times 10^2\) | 5 \(\times 10^2\)–5 \(\times 10^4\) | [213] |
| AuNPs@ g-C\(_3\)N\(_4\) |                              | Ovarian cancer | phosphatidylserine | 120 | 39 | 1 \(\times 10^2\)–1 \(\times 10^7\) | [216] |

**Abbreviations:** BPNSs, black phosphorus nanosheets; AuNPs, gold nanoparticles; MOFs, metal organic frameworks; COFs, covalent organic frameworks; Au-NPFe\(_2\)O\(_3\)NC, gold-loaded ferric oxide nanocubes; PB, Prussian blue; QDs, quantum dots; PDA, polydopamine; BPQDs, black phosphorus quantum dots; EGFR, epidermal growth factor receptor; EGFRvIII, epidermal growth factor receptor variant (v) III mutation; PD-L1, programmed death ligand-1; PLAP, placental alkaline phosphatase; EpCAM, epithelial cell adhesion molecule; GPC-1, glypican-1.
surface of superparamagnetic NPs and then hybridized with two ssDNA strands with the decreased hybridization energy. Exosome bound to the aptamer with high affinity resulted in the release of double amounts of ssDNA to initiate the amplification cycle with two hairpin DNA strands. Molecular beacon HP2 was opened and the fluorescence of probe was recovered. The two released ssDNA sequences with low concentration initiated the amplification cycle with two hairpin DNA strands. Numerous molecular beacon HP2 were opened and the fluorescence of probe was recovered. The detection limit of this method was 100 particles/μL for the assay of urine samples.

In contrast to traditional fluorescence dyes, QDs possess size/component-tunable luminescence and excellent resistance against photobleaching, which have been widely applied in bioimaging, luminescent bio-label and light-emitting devices. Wu et al proposed a “one-step” strategy for the detection of exosomes using aptamers as the biorecognition elements and QDs as the signal-amplified reporters (Figure 2A). CD63 aptamer was anchored on the surface of magnetic microspheres (MMs) and tethered to the self-assembled DNA concatemers. Then, streptavidin (SA)-conjugated QDs were used to label the biotin-modified DNA concatemers. Exosome preferentially bound to the aptamer and induced the release of QDs-labeled DNA concatemers. After the magnetic separation, the fluorescence signal in the supernatant was monitored for exosome detection. Zhang et al prepared a biomimetic periodic nanostructure-based diagnostic biochip for exosome detection using QDs. As displayed in Figure 2B, glypican-1 (GPC1) antibody-modified QDs were utilized to label exosomes. When the solution was dropped on the photonic crystals-coated biochip, the fluorescence was significantly amplified. In addition, QDs-embedded silica NPs were also used to extracellular vesicles via membrane biotinylation strategy in lateral flow assay (LFA). Metal nanoclusters (such as Au, Ag and Cu) with ultrasmall size possess excellent fluorescent properties. Ye’s group proposed a copper-mediated signal-amplified method to quantify exosomes (Figure 2C). In this work, cholesterol-modified MBs were used to capture exosomes via the hydrophobic interaction between cholesterol group and lipid membrane. CD63 aptamer-modified CuO NPs were utilized to label exosome and Cu²⁺ ions were released under acidolysis after the magnetic separation. Numerous released Cu²⁺ ions could be reduced into
fluorescent Cu nanoclusters with poly(thymine) as the template. Furthermore, Lyu et al constructed a luminescent nanosensor for exosome detection by using an afterglow semiconducting polyelectrolyte nanocomplex (ASPNC). As displayed in Figure 2D, the backbone of poly(phenylenevinylene) (PPV) modified with cationic quaternary ammonium groups was conjugated with tetra-phenylporphyrin (TPP) for the red-shift of the emission and the enhancement of the afterglow signal. The positively charged ASPNC could adsorbed the quencher the black hole quencher 2 (BHQ-2)-labeled aptamer through the electrostatic interaction and the afterglow and fluorescence signal were quenched through the efficient electron transfer between the PPV backbone and BHQ-2. In the presence of exosomes, the specific and strong affinity between exosomal protein and BHQ-2-labeled aptamer resulted in the desorption of aptamer from the ASPNC. Consequently, the afterglow and fluorescence signal were restored.

Nanomaterials with excellent fluorescent quenching ability are attractive to develop “on-off” biosensors. Zhang’s reported a dual-signal amplification platform for the analysis of leukemia-derived exosomes based on rolling circle amplification (RCA) and nicking endonuclease-assisted target recycling. In addition, Yu et al proposed a 3D DNA motor-based platform for the detection of exosomes by using AuNPs as the tracks. As shown in Figure 3A, AuNPs were modified with fluorescein-labeled substrate strands and aptamer-locked motor strands. In the presence of exosomes, the aptamer bound to the target protein and the motor strand was unlocked to trigger the DNA motor process. Powered by restriction endonuclease, the motor strands autonomously walked along the track, leading to the release of many fluorescent molecules. Gold nanorods (AuNRs) with tunable aspect ratio-dependent plasmonic extinction band can also be utilized to quench the luminescence. For instance, Chen’s group reported a simple paper-supported biosensor with AuNRs to quench
the luminescence of upconversion nanoparticles (UCNPs) through luminescence resonance energy transfer (LRET). As illustrated in Figure 3B, the sequence of aptamer toward CD63 protein is divided into two flexible ssDNA pieces with different sequence (CP and DP). Branched polyethylenimine (PEI)-modified UCNPs and CP were immobilized on the surface of filter paper by the formation of Schiff base. When exosomes were added, CD63 on the surface of exosomes facilitated the combination of DP and CP together into the intact aptamer tertiary, resulting in the close of AuNRs and UCNPs. The distance between AuNRs and UCNPs was shortened to allow for the occurrence of LRET. However, with the CD63, there was no interaction between two fragments and the LRET could not occur.

Graphene oxide (GO) can interact with DNA or RNA through π-π stacking interactions and thus quench the fluorescence of dye-labeled DNA/RNA probe through FRET. It is a fascinating nanomaterial to develop DNA-based “signal on/off” fluorescent biosensors for exosome detection. Wang et al designed a DNase I enzyme-aided signal amplification strategy for fluorescence analysis of colorectal cancer (CRC) exosomes based on the interaction between GO and aptamer. As illustrated in Figure 4A, the fluorescence of two aptamers (CD63 and epithelial cell adhesion molecule or EpCAM) labeled with different fluorophores was quenched by GO. In the presence of exosomes, two aptamers were bound to the target proteins of CD63 and EpCAM on the surface of CRC exosomes and then released from the surface of GO. DNase I promoted the digestion of the aptamers and induced the release of exosomes to liberate more aptamers, thus achieving a signal amplification. Few exosomes resulted in the release of numerous dyes and the restoration of fluorescence. Li et al developed a homogeneous magneto-fluorescent nanosensor for exosome analysis using GO as the quencher to reduce the background signal. As shown in Figure 4B, after exosomes were isolated by GPC-1 antibody-coated MBs, an extended CD63 aptamer was used to label the exosomes and the extended terminus served as a toehold to initiate the strand displacement, resulting in the formation of a large number of DNA three-way junctions (TWJ). After the magnetic separation, DNA TWJ in the supernatant could adsorb numerous positively charged derivatives of tetraphenylethene (TPE) aggregation-induced emission luminogens (AIEgens) through the electrostatic interaction. As a result, an enhanced fluorescence signal was observed. Meanwhile, GO was added to quench the fluorescence of AIEgens-stained ssDNA. The novel method achieved a wide linear detection range and the detection limit was calculated to be 6.56×10^4 particles/μL. In addition, MoS₂-multiwall carbon nanotubes nanocomposites were employed to quench the fluorescence of dye-labeled CD63 antibody, which could be restored after the immunoreaction between exosome and antibody.

As one subclass of 2D transition-metal carbides and carbonitrides materials, ultrathin MXenes have attracted much attention in biomedical applications due to their superior properties similar to those of GO. Based on their outstanding quenching efficiency, MXenes have been intensively utilized to construct fluorescent biosensors for the detection of targets, including DNA, RNA and...
proteins. Recently, Liu and co-workers reported a Ti$_3$C$_2$ MXenes-based self-standard ratiometric FRET platform for the detection of exosomes (Figure 4C). In the work, Cy3-labeled CD63 (Cy3-CD63) aptamers were adsorbed on the surface of MXenes by hydrogen-bond and metal-chelate interactions. The fluorescence of Cy3-CD63 aptamer was quenched by FRET and the intrinsic fluorescence of MXenes showed little change as a standard reference. Exosomes could specifically bind to the aptamer and induce their release from the surface of MXenes, thus leading to the recovery of fluorescence signal.

Colorimetric Biosensors

Colorimetric biosensors have attracted extensive attention because of their low cost and convenient readout. The results can be quickly observed with naked eyes. Thus, colorimetric assay is of great importance for point-of-care testing in facility-limited settings. Normally, enzymes are required to catalyze the chromogenic reaction in colorimetric assays. In traditional ELISA for the detection of exosomes, horseradish peroxidase (HRP) linked with detection antibody was always used to catalyze the reaction between H$_2$O$_2$ and colorimetric substrate 3,3′,5,5′-tetramethylbenzidine (TMB). Then, the color of solution changes from colorless to blue. However, they face the problems of low reproducibility and sensitivity (with a minimum amount of 3 μg of purified samples). Hemin/G-quadruplex with HRP-mimicking catalytic activity has also been widely used in bioassays for signal amplification. To improve the sensitivity, several strategies have been proposed for signal amplification, such as using immune-magnetic nanoparticles (MNPs) to enrich exosomes and using NPs to enhance the amount of enzymes for signal output.
reported the direct quantification of exosomes based on HCR- and HRP-mediated signal amplification.\textsuperscript{116} As shown in Figure 5A, after exosomes were captured by CD9 antibody-coated MBs, the bivalent-cholesterol-labeled DNA probes were added to recognize the lipid membrane of exosomes via the hydrophobic cholesterol moiety. The DNA probe triggered the HCR and then numerous SA-HRP conjugates were captured by the DNA polymer for catalyzing the chromogenic reaction. The proposed assay showed a detection limit of $2.2 \times 10^3$ particles/μL. In addition, DNA nanoflowers were also employed to encapsulate HRP, thus improving the loading number of enzyme in signal output.\textsuperscript{117} Yang et al proposed a pH-responsive paper-based bioassay for the detection of exosomes.\textsuperscript{118} As shown in Figure 5B, after the SA-coated MNPs-based capture of exosomes, HRP conjugated with CD63 antibody catalyzed the formation of polydopamine film on the exosome surface, thus allowing for the binding of ureases.\textsuperscript{119} The captured ureases could hydrolyze urea into ammonia and carbon dioxide, resulting in the change of solution pH value from 5 to 10 and the color change of commercially available pH test paper. However, the utilization of natural enzyme is confronted of severe disadvantages of low stability, high-cost and complicated preparation process.

Au and Ag NPs with local surface plasmon resonance (LSPR) characteristics exhibit higher extinction coefficient than the organic chromogens. Such NPs have been widely used as the alternative substrates to develop plasmonic colorimetric methods for bioassays. The detection principles of NPs-based colorimetric strategies can be divided into two subclasses: aggregation/disaggregation and etching/growth. Typically, Maiolo et al presented a simple plasmonic colorimetric strategy for the determination of exosomes.\textsuperscript{120} As shown in Figure 6A, AuNPs could aggregate at the lipid membrane of exosomes, resulting in the shift and broaden of LSPR absorption spectrum and the change of solution color from red to blue. However, in the presence of exosome and protein contaminants, the formation of protein corona around AuNPs prevented the aggregation, and the LSPR absorption kept unchanged. Tan and co-workers developed a colorimetric aptasensor for profiling of exosomal proteins (Figure 6B).\textsuperscript{121} In this work, conjugation of aptamer with AuNPs prevented NPs from aggregation in high salt solution. However, the specific interaction between aptamer and exosome made aptamer leave the surface of AuNPs and resulted in the aggregation of AuNPs with the color change from red to blue. Liu et al reported a rapid and convenient colorimetric method for the detection of exosomes at ultralow concentrations by integrating target-induced proximity ligation assay (PLA) with recombinase polymerase amplification (RPA) and transcription-mediated amplification (TMA).\textsuperscript{122} As illustrated in Figure 6C, after two PLA probes bound to the protein LMP1 on the surface of exosomes, two DNA probes hybridized with each other. Under the RPA and TMA amplification, multiple copies of RNA transcripts were generated, which could induce the aggregation of the DNA-modified AuNPs and the change of solution color. Furthermore, the principle of AuNPs-based color assay have been introduced into LFA for rapid and sensitive analysis of exosomes.\textsuperscript{123–126} In addition, because AuNR is more sensitive to the change of local media environment, and the AuNR-based color change is more realistic, AuNR-based colorimetric assays exhibit better
Zhang et al reported the multicolor visual assay of exosome by the enzyme-catalyzed metallization of AuNR and HCR amplification. As depicted in Figure 7A, after exosomes were captured and labeled with cholesterol-modified DNA probes, the terminal DNA probes initiated the HCR assembly. Large numbers of alkaline phosphatase (ALP) molecules were loaded on the exosome surface to catalyze the production of ascorbic acid (AA) and the in-situ formation of silver shells on AuNRs, alongside with a vivid change of solution color. Moreover, Au nanobipyramid@MnO$_2$ nanosheet was also used as the substrate for colorimetric detection of exosomes. During the exosome-induced competitive reaction, a large amount of ALP molecules were released into the solution by few exosomes and the free ALP catalyzed the generation of AA to etch Au nanobipyramid@MnO$_2$ nanosheet, accompanied with multicolor change. Since Fe$_3$O$_4$NPs were reported to show peroxidase-like activity, more and more nanomaterials called nanozymes have proven to show catalytic ability and have been integrated into colorimetric bioassays. Compared to the natural enzymes, the nanozymes show attractive advantages of improved stability, low cost and ease of storage. Surface charge and composition are two crucial roles to

**Figure 6 (A)** Schematic of nanoplasmonic assay for probing by eye protein contaminants (single and aggregated exogenous proteins, SAP) in EV preparations. Reprinted with permission from Maiolo D, Paolini L, Di Noto G, et al. Colorimetric nanoplasmonic assay to determine purity and titrate extracellular vesicles. *Anal Chem.* 2015;87:4168–4176. Copyright 2015 American Chemical Society. **(B)** Schematic of the displacement of aptamers from gold nanoparticles by binding with exosome surface protein and the concomitant aggregation of gold nanoparticles. **(C)** Schematic of the PLA-RPA-TMA assay. Reprinted with permission from Liu W, Li J, Wu Y, et al. Target-induced proximity ligation triggers recombinase polymerase amplification and transcription-mediated amplification to detect tumor-derived exosomes in nasopharyngeal carcinoma with high sensitivity. *Biosens Bioelectron.* 2018;102:204–210. Copyright 2018 Elsevier B.V.
regulate the catalytic activity of nanozymes. ssDNA can improve the peroxidase-mimicking activity of nanozymes.\(^\text{130}\) Chen et al reported the colorimetric assay of exosomes through ssDNA aptamer-enhanced peroxidase activity of Fe\(_3\)O\(_4\)NPs (Figure 7B).\(^\text{131}\) They found that aptamers attached on the surface of Fe\(_3\)O\(_4\)NPs could increase the affinity between NPs and TMB, thus leading to the enhancement of peroxidase activity. In the work, anion exchange method was first designed to extract exosomes from plasma. Then, the captured exosomes bound to aptamers from NPs and led to the decrease in the catalytic activity. Moreover, Wang et al developed the ssDNA-enhanced nanozyme-based colorimetric method for exosome detection (Figure 7C).\(^\text{132}\) They demonstrated that ssDNA could accelerate the intrinsic peroxidase-mimicking activity of graphitic carbon nitride nanosheets (g-C\(_3\)N\(_4\) NSs) through the electrostatic and aromatic stacking interactions between ssDNA and TMB. However, CD63 on the surface of exosomes could competitively bind with ssDNA aptamer and reduce the enhancement of the peroxidase-mimicking activity. The method is sensitive and could determine exosomes in the range from 1.9×10\(^6\) to 3.38×10\(^7\) particles/μL. Xia et al employed ssDNA-modified single-wall carbon nanotubes for the detection of exosomes by the same principle.\(^\text{133}\) On the contrary, Zhang et al reported the label-free colorimetric assay of exosomes based on ssDNA-modulated oxidase-mimicking activity of CuCo\(_2\)O\(_4\) nanorods. (Figure 7D).\(^\text{134}\) As shown in Figure 7D, CuCo\(_2\)O\(_4\) NRs could catalyze the oxidation of ABTS with O\(_2\) as the electron acceptor, instead of volatile H\(_2\)O\(_2\). The negatively charged CD63 aptamers were adsorbed on the CuCo\(_2\)O\(_4\) NRs through the electrostatic interaction and the oxidase-like activity of NRs was inhibited by hindering the electron transfer between NRs and

Figure 7 (A) Schematic of the mechanism for multicolor visual detection of exosomes based on HCR and enzyme-catalyzed metallization of Au NRs. Reprinted with permission from Zhang Y, Wang D, Yue S, et al. Sensitive multicolor visual detection of exosomes via dual signal amplification strategy of enzyme-catalyzed metallization of Au nanorods and hybridization chain reaction. \textit{ACS Sens.} 2019;4:3210–3218. Copyright 2019 American Chemical Society.\(^\text{127}\) (B) Schematic of the visible detection of exosomes based on ssDNA-enhanced Fe\(_3\)O\(_4\) NPs nanozyme activity. Reprinted with permission from Chen J, Xu Y, Lu Y, Xing W. Isolation and visible detection of tumor-derived exosomes from plasma. \textit{Anal Chem.} 2018;90:14207–14215. Copyright 2018 American Chemical Society.\(^\text{131}\) (C) Schematic of DNA aptamer accelerating the intrinsic peroxidase-like activity of g-C\(_3\)N\(_4\) NSs for the detection of exosomes. Reprinted with permission from Wang YM, Liu JW, Adkins GB, et al. Enhancement of the intrinsic peroxidase-like activity of graphitic carbon nitride nanosheets by ssDNAs and its application for detection of exosomes. \textit{Anal Chem.} 2017;89:12327–12333. Copyright 2017 American Chemical Society.\(^\text{132}\) (D) Schematic of the detection mechanism for the visible detection of exosomes based on ssDNA-enhanced Fe\(_3\)O\(_4\) NPs nanozyme activity. Reprinted with permission from Zhang Y, Su Q, Song D, Fan J, Xu Z. Label-free detection of exosomes based on ssDNA-modulated oxidase-mimicking activity of CuCo\(_2\)O\(_4\) nanorods. \textit{Anal Chim Acta.} 2021;1145:9–16. Copyright 2021 Elsevier B.V.\(^\text{134}\)
substrates. However, in the presence of exosomes, aptamers were released from the CuCo$_2$O$_4$ NRs and the oxidase-like activity was restored.

Surface Plasmon Resonance Biosensors

Surface plasmon resonance (SPR) is a label-free, real-time sensing technique to study and quantify biomolecular interaction. It can monitor the change of refractive index in close proximity to the gold surface (within 200 nm), resulting from binding event-induced increase of thickness. Moreover, it possesses the merits of high signal-to-noise, good compatibility with microfluidic technique and advanced surface modifications. Therefore, exosomes with around 100 nm size and large mass can be detected by SPR, whose size fits within the surface plasmon wave depth. To date, a series of label-free SPR biosensors for exosome detection have been developed by modifying the sensor surface with antibodies specific to the membrane proteins on exosome. To overcome the slow diffusion-limited mass transfer, magnetic nanoparticles can be utilized to pre-concentrate exosomes on the sensor surface under an external magnetic field gradient. However, the poor sensitivity of the methods limits their further applications for analyzing trace targets in complex samples.

AuNPs can enhance the SPR signal through plasmonic coupling. Wang et al proposed a SPR aptasensor for quantification of cancerous exosomes with dual AuNPs-assisted signal amplification. As illustrated in Figure 8, the gold chip was functionalized with aptamers to capture exosomes. Aptamer/T$_{30}$-modified AuNPs were further used to label exosomes on the Au film. The sensitivity of

Figure 8 Schematic of dual AuNP-assisted signal amplification for SPR determination of exosomes. Reprinted with permission from Wang Q, Zou L, Yang X, et al. Direct quantification of cancerous exosomes via surface plasmon resonance with dual gold nanoparticle-assisted signal amplification. Biosens Bioelectron. 2019;135:129–136. Copyright 2019 Elsevier B.V.}

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the biosensor is very high because of the electronic coupling between the gold film and AuNPs. For increasing the specificity, they further developed a dual-aptamers-based SPR strategy for the determination of human hepatic carcinoma (SMMC-7721) exosomes. In addition, combining with DNA-based signal amplification, Ding’s group reported a hydrogel-AuNP supramolecular sphere (H-Au)-based SPR biosensor for analysis of prostate cancer-derived exosomes. The H-Au network was prepared via the self-assembly of DNA chains and DNA-modified AuNPs. The PSMA-specific aptamer-functionalized MBs were used to transduce target exosomes into triple DNAs, which could initiate the terminal transferase-catalyzed generation of polyA tails than can bind to the H-Au network on the SPR surface. This biosensor had a wide linear range from 1.00×10^2 to 1.00×10^4 particles/μL.

### Surface Enhanced Raman Scattering Biosensors

Raman spectroscopy can provide a characteristic fingerprint spectrum. However, the signal intensity is always too weak to be distinguished. Since SERS effect was discovered, a significant interest in SERS study was aroused. Generally, metal nanostructures or nanomaterials can be employed to amplify the signal through chemical and electromagnetic field enhancement. SERS spectroscopy has been used for the design of biosensors by label-free analysis and SERS-tag-based methods.

Label-free SERS analysis is mainly based on the use of roughened or nanosized SERS substrate to enhance the weak Raman vibration signal of exosomal biomolecules with fingerprint characters. For example, Avella-Oliver et al reported the label-free SERS analysis of exosomes with large-scale substrates from recordable compact disk by coating it with silver. This cost-effective technology provided an alternative solution to perform SERS biosays in non-specialized environments. Inspired by the concept of beehives, Dong et al suggested that the Au-coated TiO2 macroporous inverse opal (MIO) structures could be used as the SERS substrates for label-free detection of exosomes. As shown in Figure 9A, different from traditional SERS substrates, the MIO structure could capture exosomes by its interconnected nanopore networks, exhibiting a prominent “slow light effect” and enhancing the Raman signal of exosomes by the SERS effect of Au layer. The SERS intensity of 1087 cm⁻¹ from the P-O bond within phosphoproteins on the surface of exosomes was used as the detection criterion. Because of the heterogeneity, the Raman spectra of exosomes show complex and inconsistent data, which are difficult to be classified. For this view, principal component analysis was employed to monitor the Raman signal, and a meaningful pattern for exosome analysis was obtained.

Au and Ag nanomaterials with LSPR have been utilized as the SERS-active nanotags to enhance the signal intensity of Raman dyes. With immunomagnetic beads and for the capture of exosomes, several aptamer or antibody-modified SERS nanotags have been developed for exosome detection via the formation of antibody-exosome-aptamer sandwich-type immunocomplexes. Microfluidic Raman biochips were also fabricated to isolate and determine exosomes in situ. Typically, Wang’s group reported an effective approach for the detection of exosomes by simultaneously profiling multiple protein biomarkers on the surface. As shown in Figure 9B, three specific nanotags for antibody modification were prepared and labeled in the filtered conditional exosome-suspension medium. Then, the antibody-modified CD63-conjugated MBs were added for the sandwich-like immunoassays. The heterogeneous antigens expressed on diverse exosomes limited the applications of the methods based on the antigen-antibody/aptamer interaction. Liu’s group proposed a general, facile, and robust strategy to label exosomes with maleimide (Mal) tags by the hydrophobic insertion. As shown in Figure 9C, maleimide-terminated DSPE-PEG (DSPE-PEG-Mal) as labeling probe was inserted into the lipid membranes. Male group could be conjugated with the thiol-containing species (1,6-hexanedithiol) via the click chemistry and further bound to bare AuNPs for SERS analysis. Wang’s group developed a SERS biosensor for multiple assays of exosomes with gold layer-coated MBs as SERS probes which were modified with three different types of Raman reporters and aptamers. Besides, Kwizera et al presented a method for exosome detection by using cationic AuNRs as SERS tags to label exosomes through the electrostatic attraction. The “hot spots” generated in the AuNP-AuNP junctions due to the plasmon coupling effect can intensify the Raman signal of SERS molecules. For this view, Ning et al reported the multiple SERS assays of exosomes using gold-silver bimetallic nanotrengs, in which different Raman reporter molecules were confined in the interfaces of gold core and silver shell. Zhang et al designed a novel Raman probe for the assays of exosomes by assembling AuNPs in triangular pyramid DNA (TP-DNA). As illustrated in Figure 9D, TP-DNA was
prepared through the hybridization of four X-shaped DNA sequences and then the positively charged AuNPs and Raman reporter molecules were entrapped through the electrostatic interactions. Besides, laser-tweezers Raman spectroscopy has also been employed to determine exosomes with individual nanoparticle for signal enhancement.

**Nanomaterials-Based Electrochemical Biosensors for Exosome Detection**

Electrochemical biosensor has been recognized as an excellent platform for biological sample analysis due to its advantages of high sensitivity, low cost, rapid response and low sample volume. Several classic electrochemical techniques are frequently utilized in bioassays, including amperometry, voltammetry, impedimetry and field effect transistor. Nanomaterials mainly play two vital roles in these techniques: as the electrode substrate to improve the electron transfer and as the functional nano-tags for signal amplification.

**Direct Detection**

Direct electrochemical detection is achieved by monitoring the change of electrical conductivity of electrode by the target-induced electrical signal change. The method can quantify exosomes without labeling step, thus shortening the response time. For example, Tan and co-workers presented an electrochemical aptasensor for direct determination of cancerous exosomes by using DNA nanotetrahedra to immobilize aptamer on the electrode surface to improve...
the accessibility of exosomes. Davis’s group reported an immunosensor for the analysis of exosomes by electrochemical impedance spectroscopy. Vaidyanathan developed a multiplexed device to detect exosomes by alternating current electrohydrodynamic induced nanoshearing.

AuNPs with good electrical conductivity and easy of functionalization have been widely utilized to modify the sensor electrode. Cucurbit[7]uril with excellent supramolecular recognition ability toward ferrocene (Fc) has been widely used as the receptor in electrochemical analysis. Liu et al reported a label-free electrochemical aptasensor for exosome detection based on the host-guest interaction between cucurbit[7]uril and Fc. As illustrated in Figure 10A, cucurbit[7]uril was immobilized on the AuNPs-modified electrode for the capture of Fc-labeled CD63 aptamer. The aptamer bound to the target exosome with high affinity can be released from the electrode surface, thus resulting in the decrease of electrochemical signal. Sun et al developed a dual-signal and intrinsic self-calibration aptasensor for direct detection of exosomes. As shown in Figure 10B, ITO slice was modified with Fc-doped metal-organic frameworks (ZIF-67) by electrodeposition and black phosphorus nanosheets (BPNSs). Then, the methylene blue-labeled ssDNA aptamers were adsorbed on the electrode surface. The platform exhibited dual redox-signal responses from methylene blue and Fc. In the presence of exosomes, the aptamers were desorbed from the electrode surface, leading to the decrease of redox current of methylene blue. In this process, no significant change was observed for the current of Fc. The intrinsic self-calibration aptasensor showed a detection limit down to 0.1 particles/μL.

Field effect transistor (FET) biosensor is a promising label-free detection tool, which can monitor the microelectrical signal caused by the interaction between target and recognition element on the sensing interface. Yu et al designed a reduced graphene oxide (rGO)-based FET biosensor for electrical and label-free quantification of exosomes. In this paper, 1-pyrenebutanoic acid succinimidyl ester was modified on the rGO surface through the π-π stacking interaction between pyrene and graphene. Then, CD63 antibody was covalently immobilized on the FET surface. The net carrier density changed with the introduction of negatively charged exosomes, thus leading to the left shift of the Dirac point.

Sandwich-Like Methods

Although the label-free electrochemical method is simple, it shows poor sensitivity and selectivity. Therefore, different types of sandwich-like methods were developed for bioassays. Generally, the electrode was modified with a biorecognition element to capture exosome, and the another biorecognition element modified with a signal reporter was added to recognize the captured exosome and produce an electrical signal. Enzymes and electroactive molecules are usually used as the signal reporters for signal amplification. For example, Doldan et al reported an electrochemical immunosensor for the determination of exosomes using HRP-conjugated antibody. An et al designed an electrochemical aptasensor for the detection of tumor exosomes by the HCR assembly to linking numerous HRP molecules for catalytic redox...
reactions. He and co-workers reported an electrochemical aptasensor for the analysis of exosomes by hemin/G-quadruplex-assisted rolling circle amplification. Generally, nanomaterials can act as nanocarriers, nanoelectrocatalysts and electroactive tags for signal amplification in sandwich-like electrochemical assays.

Because of their excellent biocompatibility and large surface area, AuNPs have been widely used to carry various biomolecules (such as proteins, DNA and RNA) for different biological applications. Jiang et al. reported an electrochemical aptasensor for exosome detection using AuNPs and enzyme for signal amplification. As showed in Figure 11A, aptamer-modified DNA nanotetrahedron (NTH) was employed to modify the electrode, avoiding the problem of the entanglement of aptamer and the spatial hindrance effect. Aptamer and biotin were modified on AuNPs through the interaction between polyA10 and AuNPs. After the formation of sandwich-like complexes, numerous avidin-HRP conjugates were immobilized on the biotin-labeled AuNPs to catalyze the reduction of TMB and H2O2.

Due to their high specific surface area, flexible porosity, and tunable framework structure, metal–organic frameworks (MOFs) have attracted wide attention in comprehensive applications, including catalysis, sensors and energy conversion and storage. The porosity endows MOFs with the ability to carry plenty of enzyme or electroactive molecules. Sun et al. reported a label-free and enzyme-free electrochemical biosensor for the detection of glioblastoma-derived exosomes using Zr-based MOFs. As displayed in Figure 11B, Zr-MOFs (UiO-66) prepared from metal ions and organic ligands through a hydrothermal method were utilized to load numerous

Figure 11 (A) Schematic of the electrochemical aptasensor for exosomes detection by using AuNPs and enzyme for amplification. Reprinted with permission from Jiang J, Yu Y, Zhang H, Cai C. Electrochemical aptasensor for exosomal proteins profiling based on DNA nanotetrahedron coupled with enzymatic signal amplification. Anal Chim Acta. 2020;1130:1–9. Copyright 2020 Elsevier B.V. (B) Schematic of the fabrication process of MB@UiO-66-based nanoprobe and the electrochemical biosensor for the detection of GBM-derived exosomes. Reprinted with permission from Sun Z, Wang L, Wu S, et al. An electrochemical biosensor designed by using Zr-based metal-organic frameworks for the detection of glioblastoma-derived exosomes with practical application. Anal Chem. 2020;92:3819–3826. Copyright 2020 American Chemical Society. (C) Schematic of identification of PD-L1+ exosomes based on HRCA-responsive PVP®HRP®ZIF-8. Reprinted with permission from Cao Y, Wang Y, Yu X, Jiang X, Li G, Zhao J. Identification of programmed death ligand-1 positive exosomes in breast cancer based on DNA amplification-responsive metal-organic frameworks. Biosens Bioelectron. 2020;166:112452–112460. Copyright 2020 Elsevier B.V. (D) Schematic of the fabrication process of COFs-based nanoprobes and the mechanism of the EC biosensor for exosomes detection. Reprinted with permission from Wang M, Pan Y, Wu S, et al. Detection of colorectal cancer-derived exosomes based on covalent organic frameworks. Biosens Bioelectron. 2020;169:112638–112645. Copyright 2020 Elsevier B.V.
electroactive methylene blue molecules. After the capture of exosomes by the peptide-modified electrode, methylene blue-loaded Zr-MOFs were anchored by exosomes. Zr-MOFs interacted with phosphate groups in the phospholipid bilayer of exosomes with high affinity via the formation of Zr-O-P bonds. The concentration of exosomes could be determined by measuring the electrochemical signal of methylene blue inside of MOFs. Recently, Gu et al presented a biofuel cells-based self-powered biosensor for exosome detection, in which two types of MOFs (ZIF-8 and UiO-66-NH\textsubscript{2}) were utilized to load glucose dehydrogenase and electroactive molecules (K\textsubscript{3}[Fe(CN)\textsubscript{6}]), respectively.\textsuperscript{191} Taking the advantage of exogenous stimulus-responsive property, Cao et al reported an electrochemical biosensor for the detection of programmed death ligand-1 positive (PD-L1\textsuperscript{+}) exosomes based on DNA amplification-responsive MOFs.\textsuperscript{192} As illustrated in Figure 11C, HRP-encapsulated ZIF-8 was prepared by biomineralization-facilitated method and then coated with PVP, which remained intact at weak alkaline pH and would be broken at acidic pH. After the capture of PD-L1\textsuperscript{+} exosomes by immune-MBs, the anti-PD-L1-linked DNA strands were added to label exosomes and the DNA parts initiated the hyperbranched rolling circle amplification (HRCA). The released H\textsuperscript{+} ions caused the change of the environment pH to weak acidity, thus leading to the disassembly of MOFs. HRP molecules were released to increase the electrochemical response.

As an emerging kind of porous crystalline materials, covalent organic frameworks (COFs) exhibit great application potential in bioassays. Li’s group reported a COFs-based aptasensor for the analysis of CRC exosomes.\textsuperscript{193} As shown in Figure 11D, spherical COFs with high porosity were utilized to load para-sulfocalix[4]arene hydrate (pSC\textsubscript{4})-modified AuNPs and a large amount of HRP molecules to form HRP-pSC\textsubscript{4}-AuNPs@COFs. pSC\textsubscript{4} could specifically bind with various amino acid residues on the exosome surface. AuNPs could accelerate the charge transfer of carriers. CD63 aptamer was anchored on the surface of Au electrode to capture exosomes. Then, HRP-pSC\textsubscript{4}-AuNPs@COFs were added to recognize the captured exosomes and HRP catalyzed the oxidation of TMB by H\textsubscript{2}O\textsubscript{2}, generating a high electrochemical signal.

Nanozymes can catalyze the redox reaction between H\textsubscript{2}O\textsubscript{2} and substrate in colorimetric assays, which can be converted into electrochemical assays with improving sensitivity. For example, Boriachek et al reported the direct isolation and subsequent detection of exosomes using gold-loaded ferric oxide nanocubes (Au-NPFe\textsubscript{2}O\textsubscript{3}NC).\textsuperscript{194} As shown in Figure 12A, the Au-NPFe\textsubscript{2}O\textsubscript{3}NC was...
modified with CD63 antibody for the capture of exosomes. After magnetic separation, the complex of Au-NPFe$_2$O$_3$NC and exosomes was attached on the placental alkaline phosphatase (PLAP) antibody-functionalized screen-printed electrode. The signal was measured by the Au-NPFe$_2$O$_3$NC-catalyzed reaction between TMB and H$_2$O$_2$.

Wang and co-workers developed a sensitive electrochemical biosensor for exosome detection through the in-situ generation of electroactive Prussian blue (Fe[Fe(CN)$_6$]) on the surface of MXenes. As illustrated in Figure 12B, the CD63 aptamer-modified poly(amidoamine)-AuNP electrode was used to capture exosomes. Then, aptamer-conjugated MXene was used to recognize the captured exosomes. MXene on the exosome surface served as a reducing carrier to induce in-situ generation of Prussian blue, simplifying the synthesis process. Prussian Blue could produce electrochemical signal at a low potential without the interference of electroactive species. The detection limit of this method was 229 particles/μL. QDs with a large amount of metal ions can also be utilized as the signal transduction labels for the assays of exosomes. After acid-assisted dissolution, numerous Cd$_{25+}$ ions were released, which could be quantified by anodic stripping voltammetry (ASV). Moreover, metal NPs such as Ag and Cu can be utilized as the signal reporters because they can be directly electrochemically oxidized to produce a typical electrochemical peak. Kelley’s group reported the electrochemical detection of exosomes/microsomes with anti-EpCAM aptamers-modified AgNPs and anti-PSMA aptamers-modified CuNPs (Figure 12C). After exosomes and microsomes were captured from VCaP cells through a simple centrifugation procedure, aptamers-functionalized NPs were added to label the captured exosomes. Then, linear sweep voltammetry (LSV) was used for the direct electrochemical oxidation of AgNPs or CuNPs.

**Magneto-Electrochemical Detection**

The immobilization of recognition probes on the electrode may suppress the effective recognition between exosome and probe and thus decrease the sensitivity. MBs have been widely used for the capture of exosomes and can be integrated into electrochemical biosensors. MBs can not only simplify the detection procedures, but also concentrate the captured exosomes on the electrode. MBs modified with antibody (immune-MBs) have been used to isolate and enrich exosomes. MBs-based electrochemical techniques for immobilization-free detection of exosomes have been developed. Lee’s group designed an integrated immuno-magneto-electrochemical sensor for exosome detection (Figure 13A), in which MBs were modified with CD63 antibodies to directly capture exosomes in plasma. Next, the captured exosomes were recognized by the HRP-labeled detection antibodies. HRP could catalyze the reaction between TMB and H$_2$O$_2$, thus generating a strong electrochemical signal. To meet the need of portability and sensitivity, Ye’s group reported a two-stage magnetic-based microfluidic platform for on-chip isolation and detection of exosomes. As illustrated in Figure 13B, a staggered Y-shaped micropillar mixing pattern was applied to create an anisotropic flow for improving the capture efficiency. Tumor-derived exosomes captured by Tim4-coated MBs were immobilized on the ITO electrode. The ssDNA in a hairpin structure consisted of aptamer and mimicking DNAzyme sequence was employed to label exosomes. After the recognition, hairpin was opened and a G-quadruplex formed with hemin was utilized as NADH oxidase and HRP-mimicking DNAzyme simultaneously. In addition, CdSe QDs were utilized as the signal labels instead of unstable enzymes for exosome detection by an anodic stripping voltammetry.

Owing to the flexible structure, aptamer can hybridize with other DNA sequences that can initiate the DNA-based signal amplification or DNA nanomachines. Therefore, exosome detection can be converted into the analysis of DNA, whose amount is proportional to the number of exosomes. This strategy avoids the direct detection of exosomes on the electrode and many methods can be developed to sensitively determine DNA. For instance, Dong et al reported a highly sensitive electrochemical biosensor for exosome detection based on aptamer recognition-induced release of multi-DNA and cyclic enzymatic amplification. As shown in Figure 14A, aptamer-messenger DNA (mDNA) complexes were first modified on the MBs. LNCaPcell-derived exosomes bound to aptamer with high affinity, resulting in the release of three kinds of mDNA sequences. After the magnetic separation, the released mDNA in the supernatant initiated the Exo III-assisted cyclic enzymatic amplification reaction, leading to the sharp decrease in the amount of Ru(NH$_3$)$_6$$^{3+}$ on the surface of Au electrode. Zhao et al reported a ratiometric electrochemical biosensor for the detection of exosomes by target-triggered 3D DNA walker and Exonuclease III-assisted cyclic enzymatic amplification. As displayed in Figure 14B, MBs were modified with high-density of DNA as 3D DNA walker...
The DNA sequence consisted of CD63 aptamer and DNAzyme substrate. In the presence of exosomes, the recognition of CD63 aptamer on the MBs and EpCAM aptamer on the swing arm simultaneously bound to different target proteins on the same exosome, leading to the close proximity effect between DNAzyme and substrate. After the hybridization, DNA walkers were initiated and a large number of oligonucleotide fragments were released, which could be sensitively detected by the Exonuclease III-assisted cyclic enzymatic amplification. To improve the capture efficiency, dual aptamers-modified MBs were employed to efficiently capture exosomes. As illustrated in Figure 14C, after the capture of tumor exosomes, the cholesterol-modified DNA probe was anchored on the exosome membrane through the hydrophobic interaction, triggering DNA tetrahedron-based hyperbranched HCR to generate sandwich complexes. The complexes could sequestrate a large amount of Ru(NH$_3$)$_6^{3+}$ by electrostatic interactions, thus reducing the amount of Ru(NH$_3$)$_6^{3+}$ in the solution after the magnetic separation. This caused the change of current ratio of [Fe(CN)$_6$]$_3^{3-}$ to Ru(NH$_3$)$_6^{3+}$.

### Electrochemiluminescent Biosensors

As a powerful technique, ECL has been applied in various bioanalysis due to the remarkable advantages of low background signal, high sensitivity and wide detection range. To meet the need of ultrahigh sensitivity, loading of numerous luminophores into nanomaterials can boost the ECL efficiency and improve the analysis sensitivity. Feng et al designed an aptamer-binding DNA walking machine for sensitive ECL detection of tumor exosomes by employing Ru(bpy)$_3^{2+}$-loaded silica NPs as the signal reporters. To date, several nanomaterials have been utilized as the ECL emitters for diverse biosensing. For example, Sheng and co-workers developed an ECL
aptasensor for the analysis of exosomes by the G-quadruplex/hemin DNAzymes-induced quenching of RCL signal of Eu^{3+}-doped CdSQDs.\textsuperscript{210} Zhang et al designed a sensitive g-C_3N_4-coated liquid metal nanoprobe-based ECL sensing strategy for exosome detection on the multivalency interface (Figure 15A).\textsuperscript{211} In the nanoprobe, Galinstan NPs accelerated the electrode transfer and suppressed the g-C_3N_4 passivation during the electrochemical reduction processes, thus enhancing the ECL signal. Moreover, the antibody-modified PAMAM-Au NPs were used to modify the electrode for multivalent recognition of exosomes with high capture efficiency. This method achieved a detection limit of 31 particles/μL for the determination of HeLa cell-derived exosomes. To simplify the operation procedure and reduce the contamination, Guo et al reported the QDs-based homogeneous ECL sensing of exosomes with stimuli-responsive DNA microcapsules and target recycling system.\textsuperscript{212} As illustrated in Figure 15B, Cds QDs-loaded CaCO_3 microcapsules were sealed by DNA-assembled stimuli-responsive shell layers. Then, the core CaCO_3 was removed by treatment with EDTA and the DNA shell-coated CdS QDs microcapsules were formed. The presence of exosomes initiated the nicking endonuclease (Nt.BbvCI)-assisted target recycling and the crosslinked DNA shells were disintegrated. The released QDs were determined by ECL technique. The established method with dual-amplification exhibited a wide detection range of 5×10^4 to 1×10^8 particles/μL.

Nanomaterials with excellent catalytic properties have been increasingly utilized in the development of ECL biosensors. For example, MXenes have been concerned in the areas of catalysis, biosensor and supercapacitors, owing to their excellent electron transfer ability and unique catalytic ability. Wang and co-workers reported a MXenes-catalyzed ECL biosensor for the assay of exosomes.\textsuperscript{213} Fang et al proposed a self-enhanced ECL and photothermal dual-mode biosensor for exosome detection.\textsuperscript{214} As shown in Figure 15C, MXenes were employed to carry black phosphorus (BP) quantum dots (BPQDs), Ru(dcbpy)_3^{2+} and CD63 antibody. In the nanocomposite, BPQDs not only catalyzed the oxidization of Ru(dcbpy)_3^{2+}, but also acted as the coreactants. The integration of BPQDs and Ru(dcbpy)_3^{2+} into MXenes could shorten the distance and amplify the ECL signal.

Figure 14 (A) Schematic of the highly sensitive electrochemical biosensor for exosomes detection based on aptamer recognition-induced multi-DNA release and cyclic enzymatic amplification. Reprinted with permission from Dong H, Chen H, Jiang J, Zhang H, Cai C, Shen Q. Highly sensitive electrochemical detection of tumor exosomes based on aptamer recognition-induced multi-DNA release and cyclic enzymatic amplification. Anal Chem. 2018;90:4507–4513. Copyright 2018 American Chemical Society.\textsuperscript{205} (B) Schematic of the ratiometric electrochemical biosensor for the detection of exosomes by target-triggered 3D DNA walker and Exo III-assisted cyclic enzymatic amplification. Reprinted with permission from Zhao L, Sun R, He P, Zhang X. Ultrahsensitive detection of exosomes by target-triggered three-dimensional DNA walking machine and exonuclease III-assisted electrochemical ratiometric biosensing. Anal Chem. 2019;91:14773–14779. Copyright 2019 American Chemical Society.\textsuperscript{206} (C) Schematic of the ratiometric immobilization-free electrochemical sensing system for tumor exosome detection in the absence (a) and in the presence (b) of the tumor exosomes. Reprinted with permission from Yang L, Yin X, An B, Li F. Precise capture and direct quantification of tumor exosomes via a highly efficient dual-aptamer recognition-assisted ratiometric immobilization-free electrochemical strategy, Anal Chem. 2021;93:1709–1716. Copyright 2021 American Chemical Society.\textsuperscript{207}
Moreover, both MXenes and BPQDs with good photothermal property can be used as the photothermal probes. The dual-mode biosensor exhibited a linear range of $1.1 \times 10^2$~$1.1 \times 10^7$ particles/μL. However, the stabilizers for regulating the structure and morphology and biomolecules for the specific recognition may block the active sites and hinder the electron transfer. Thus, Zhang et al reported an ECL biosensor for exosome detection based on the in-situ formation of AuNPs on the aptamer-modified MXenes (Figure 16). MXenes were modified with CD63 aptamer to identify exosomes. After the capture of exosomes, aptamer-conjugated MXenes were adsorbed on the surface of exosomes. MXenes with large surface area and strong reduction ability could induce the in-situ formation of AuNPs on their surface without the addition of extra reducing agents. AuNPs with catalytic surface greatly enhanced the ECL signal of luminol. The proposed biosensor showed a detection limit down to 30 particles/μL. In recent years, g-$C_3N_4$ was used not only as the carrier of luminol, but also as a catalyst to promote the reaction of luminol with $\text{H}_2\text{O}_2$, thus amplifying the ECL signal for exosome detection.  

**Photoelectrochemical Methods**

A photoelectrochemical (PEC) process involves in the photocurrent as the detection signal generated by photoelectrochemically active materials under light illustration. The separation of the excitation source (light) from the detection signal (electric current) endows PEC biosensors with low background signal, high signal-to-noise ratio and
excellent stability. Photosensitive materials play a vital role in the fabrication of PEC biosensors. Typically, Li’s group designed a cathodic PEC aptasensor for the detection of exosomes using p-type NiO/BiOI/AuNP composites sensitized by CdSe QDs. In this work, BiOI with a narrow bandgap (1.8 eV) was immobilized on the NiO-modified ITO to sensitize the wide band gap of NiO (3.6–4.0 eV). Then, AuNPs were deposited on the surface to conjugate EpCAM aptamers through the formation of Au-S bonds. DNA2-modified QDs were bound to the electrode surface through the hybridization, which sensitized the nanocomposite and improved the photocurrent intensity. In the presence of exosomes, aptamer bound to the EpCAM protein on the surface of exosomes, resulting in the release of QDs and the decrease of the photocurrent. Meanwhile, exosomes on the electrode surface hindered the electron transfer between electrode and electron acceptor, leading to the further attenuation of the photocurrent signal.

**Other Methods**

Chemiluminescence is generated from the exergonic chemical reaction, in which an intermediate molecule in singlet excited state undergoes radiative decay. Chemiluminescence biosensors can sensitively detect targets of interest in the dark without any extra input energy. Zhong’s group reported a chemiluminescence strategy for rapid isolation and quantification of exosomes based on CuS-enclosed microgels. As displayed in Figure 17, CuS NPs were in-situ generated in porous microgel, which further modified with streptavidin and antibody. After interaction with exosomes, microgels promoted the isolation of exosomes through membrane filtration. A large number of Cu$^{2+}$ ions were liberated and catalyzed the reaction between H$_2$O$_2$ and luminol derivate, producing strong a chemiluminescence signal. Moreover, Wang et al reported a chemiluminescence immunoassay for exosome detection by using antibody-conjugated superparamagnetic iron oxide particles (SIOPs) and acridinium ester as the signal label.

Mass spectrometer (MS) is an effective tool to characterize the content of biomolecules at high throughput. However, the low sensitivity limits its utilization in detect low concentration of exosomes at the early stage of cancers. The element-labeling strategy endow MS with the advantages of high selectivity and signal amplification. In this detection principle, nanomaterials not only act as matrix materials for exosomes capture, but also can amplify MS signal due to the large amount of elements in nanomaterials. Recently, Zhang et al reported an ultrasensitive inductively coupled plasma–mass spectrometry (ICP-MS) method for the detection of exosomes by using UCNPs as element labels. As shown in Figure 18, three different elements (Y, Eu, and Tb) doped UCNPs modified with three different aptamers were linked to AuNPs, forming three satellite-like nanoassemblies. Exosomes with surface proteins could trigger the release of the corresponding
aptamers conjugated with UCNPs from AuNPs. Then, the released UCNPs in solution were detected by ICP-MS. Finally, this sensitive and high-throughput method could distinguish exosomes from seven different cell lines.

**Conclusions**

In this review, we have systematically reviewed various nanomaterials-based techniques for exosome detection. The unique characteristics of nanomaterials make them fascinating materials for signal transduction and biosensor development. Moreover, the sensitivity and selectivity of biosensors for exosome detection have greatly improved by integration with various elaborate DNA or enzyme-based signal amplification strategies. In spite of significant progress in exosome detection, there are still some challenges to be addressed. For example, stability of nanomaterials and...
selectivity for complex samples should be improved by appropriate modification with biocompatibility polymers or others. Biorecognition elements used in bioassays may suffer from the digestion of enzymes in real samples. For transforming research in laboratory into clinical practice, it is the key obstacle to develop standardized technical specifications, including collection of biological samples, isolation of exosomes from liposiy and detection operation. It is beneficial to enhance the comparability of results and establish a reliable and comprehensive dataset for future study on exosomes. The cost-effective and high-throughput isolation and detection of exosomes is helpful to simultaneous identify a large amount of clinical samples. We believe that nanomaterials-based detection of exosomes will make greater progress with the collective and unremitting efforts of different fields such as chemistry, materials science and clinical diagnosis.

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**Disclosure**

The authors declare no conflict of interest.

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