Lipids in Health and Disease

REVIEW

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Innovative approach to the detection of circulating tumor biomarkers: multi-dimensional application of liposome technology

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Abstract

Malignant tumors represent a significant worldwide health challenge, with elevated morbidity and mortality rates necessitating enhanced early identification and individualized treatment. Liposomes, as biomimetic lipid-based nanovesicles, have developed as a multifaceted platform for detecting and treating malignant tumors due to their excellent biocompatibility, stability, and membrane fusion properties. Circulating tumor markers, such as circulating tumor cells (CTCs), extracellular vesicles (EVs), circulating tumor proteins (CTPs), and circulating tumor nucleic acids (ctNAs), play a key role in early cancer diagnosis, disease progression monitoring, and personalized therapy. Liposome-based platforms enable effective molecular recognition, targeted detection, and signal amplification by targeting circulating tumor biomarkers, significantly increasing the potential for early tumor diagnosis and treatment. This review systematically summarizes advancements in the study of liposomes concerning circulating tumor markers, including applications in targeted recognition, early detection, and disease diagnosis, while discussing present problems and prospective applications of existing technology.

Keywords Tumor, Liposomes, Circulating tumor biomarkers, Biomarker, Cancer detection

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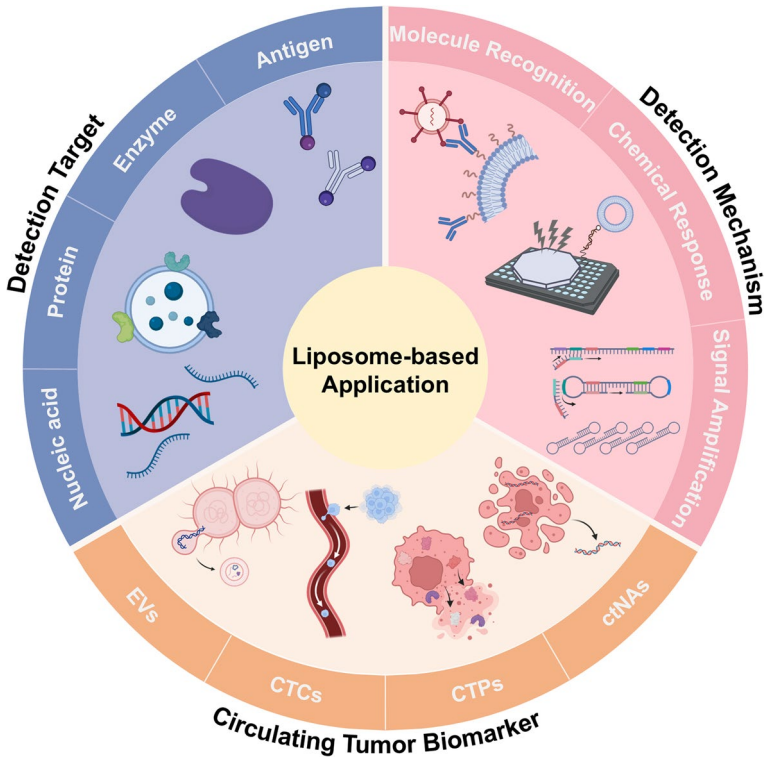
Graphical Abstract

Schematic illustration of this review's content. (Created with BioRender.com)



Introduction

Malignant tumors are a major and growing health problem worldwide, and in recent years, they have emerged as the second biggest cause of mortality, following cardiovascular disease [1], causing approximately 10 million deaths annually [2, 3]. To enhance patient survival rates, early detection and treatment are the keys to improving the prognosis of cancers and increasing the survival rate of patients. However, existing clinical diagnostic methods for tumors (such as histopathological biopsy, imaging tests, molecular tests, and immunotherapy) have shortcomings such as invasive operations, poor patient compliance, and susceptibility to experimental conditions, and there is still room for improvement in sensitivity and specificity [4, 5], which are unable to meet their broad and diverse clinical needs. Therefore, the development of sensitive, specific, non-invasive, and simple detection methods and treatment strategies with low side effects, high efficiency, and wide applicability has become a hot spot in tumor research [6].

Circulating tumor Biomarkers (CTBs) include circulating tumor cells (CTCs), extracellular vesicles (EVs), circulating tumor proteins (CTPs), and circulating tumor nucleic acids (ctNAs), which are released into the bloodstream or body fluids from tumor primary or metastatic foci [7]. These markers are highly homologous to tumors and can respond to tumor information in real time and accurately; they can be used as targets for tumor detection and treatment. CTBs-based detection techniques (such as liquid biopsy) provide many advantages, including ease of detection, identification of multiple tumor types, non-invasiveness, and identification at an early stage of pathogenesis [8], providing important support for "individualized precision medicine". However, the low abundance and heterogeneity of CTBs makes its detection in vivo difficult. To address these challenges, new strategies are being developed to improve the sensitivity and efficiency of CTB-based testing. One promising approach is the use of liposomes, which have demonstrated potential in enhancing tumor marker detection and diagnostic accuracy. Liposomes, as modifiable nanoscale vesicles, can efficiently encapsulate and deliver diagnostic agents, facilitating more precise capture and analysis of CTBs. This advances the possibilities

of overcoming current method limitations, particularly in dynamic monitoring and multi-marker detection.

These lipid-derived biomimetic nanovesicles self-assemble from concentric lipid bilayers, with a hydrophilic exterior and a lipophilic core [9]. They can encapsulate therapeutic molecules in lipid solutions, and are widely utilized as inspection systems [10, 11]. Liposomes' lipophilicity and molecular specificity allow for tumor marker detection and identification through membrane fusion, enabling rapid tumor diagnosis [12]. Recent advancements in liposome engineering, including signal cascade amplified probes, multi-target cross-linked liposome technology, nanovesicles integrated with CRISPR technology, and biomimetic liposome enhanced membrane fusion technology. These innovate methods have received significant attention for their ability to address clinical challenges such as low marker concentration, pronounced heterogeneity, and challenging to be regulated directly. Notably, liposomes can be combined with other sensing technologies, such as DNA nanotechnology [13], to further improve detection performance. A study showed that DSPE-ir623, a highly fluorescent near-infrared probe, increased the effectiveness of liposomes in cancer detection and real-time monitoring, as evidenced by HT-29 cell aggregation in tumor tissues [14]. These innovations facilitate real-time monitoring of tumor dynamics and improve the accuracy of biomarker capture and delivery, thereby addressing significant challenges in early cancer detection and promoting the shift from experimental models to clinical validation.

Herein, we summarize various liposomal strategies for capturing and detecting CTBs, while analyzing the existing challenges and prospects. Furthermore, we discuss the potential of liposomes in comprehensive diagnosis and clinical applications, aiming to provide novel scientific insights for the early diagnosis and accurate treatment of malignant cancers (Fig. 1).

Overviews of liposomes

Structural basis of liposomes

Phospholipids are the basic units that make up the structure of bilayer membrane. When phospholipids

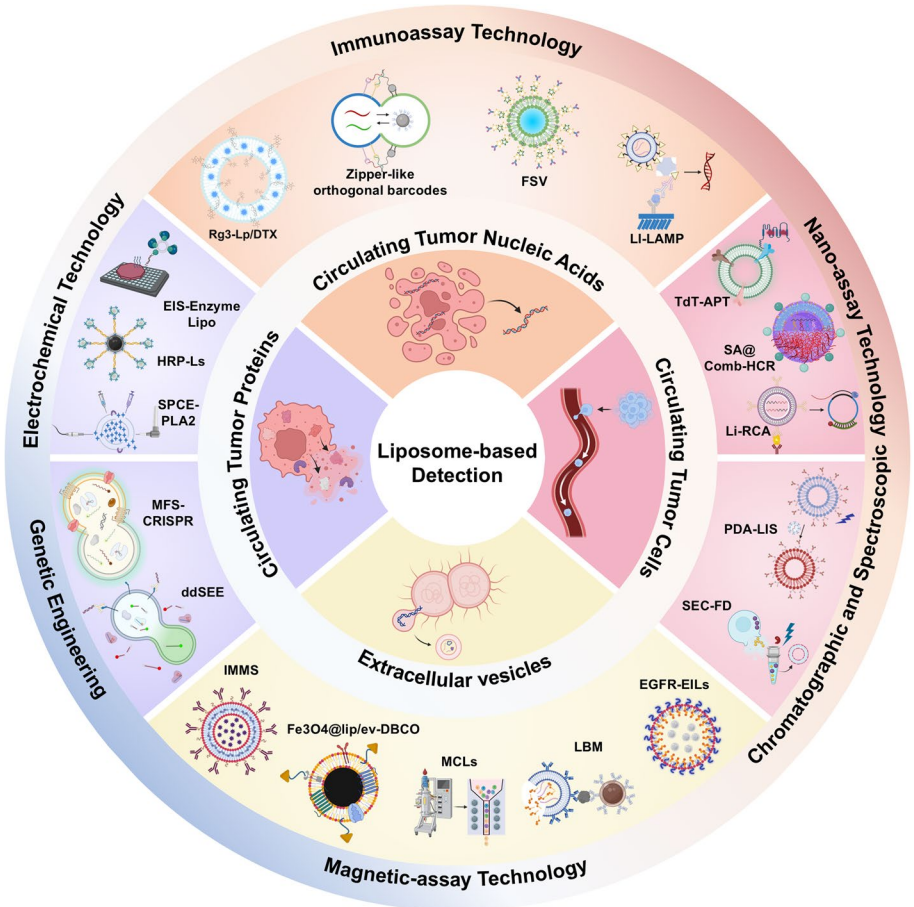


Fig. 1 Schematic illustration of the technology in CTBs detection. (Created with BioRender.com)

are dissolved in water and sufficient energy is provided to the solution by sonication, heating, homogenization, or other methods, bilayered structures are formed [15]. This self-assembly process is usually considered to be related to the critical micelle concentration (CMC), when the concentration reaches CMC, the hydrophobic effect and surfactant molecules interaction induce spontaneous arrangement of phospholipids from the original disordered soluble monolithic structure to the ordered and stable bilayered vesicle structure with minimal free energy. These biodegradable amphiphilic micellar self-assemblies are well-suited for systemic drug delivery. Their core–shell shape permits hydrophobic molecule encapsulation with sustained release and extended circulation [16]. Liposomes are currently widely used in recognition [17], drug delivery [10], therapeutic diagnostics [18], and imaging detection [19]. Liposomes with modified molecules like antibodies, ligands, or glycan chains can recognize and transport specific molecules via antigen–antibody interactions with cell surface receptors. Liposomes can also encapsulate soluble molecules and retain them within the membrane during bilayer fracture to self-enclose bilayer fragments. Modifiable membrane fluidity improves molecular stability and controls molecular release, making liposomes an appropriate carrier for molecules and probes.

Liposomes have a relatively broad size range, typically between 20 nm and several thousand nanometers. The bilayer structure is the main basis for the classification of liposomes, which can be categorized into unilamellar vesicles (ULVs) and multilamellar vesicles (MLVs) according to the size and number of bilayer structures. ULVs are single phospholipid bilayer spheres internally encapsulated in aqueous solution and can be divided into three categories: (1) small unilamellar vesicles (SUVs), with sizes ranging 20–100 nm; (2) large unilamellar vesicles (LUVs), with sizes ranging 100–1000 nm; and (3) giant unilamellar vesicles (GUVs), with sizes > 1000 nm. Multilamellar vesicles, on the other hand, have multiple lipid bilayers inside, which are concentrically or non-concentrically distributed, and are mainly categorized into (1) oligolamellar vesicles (OLVs), with sizes ranging 100–1000 nm; (2) large multilamellar vesicles (LMVs), with sizes > 500 nm; and (3) multivesicular vesicles, with sizes > 1000 nm (Table 1).

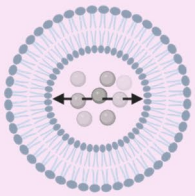
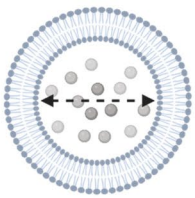
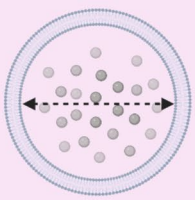
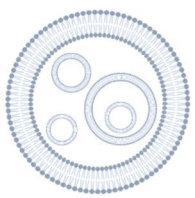
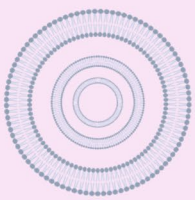
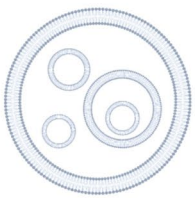
The selection of lipids in liposome production directly affects their shape, stability, and carrier capacity. Common synthetic phospholipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol [33], are known for good biocompatibility and membrane-forming ability. Cholesterol, also often used in liposome formulations, stabilizes the bilayer by reducing permeability in physiological fluids [34]. Additionally, cationic lipids like 1,2-dioleoyl-3-trimethylammonium-propane chloride (DOTAP) and dimethyldioctadecylammonium bromide (DDAB) impart positive charges to the liposome surface, generating a high zeta potential [35, 36]. This induces homomolecular repulsion and heteromolecular attraction, preventing precipitation and aggregation, while enhancing cell membrane interaction and molecular or gene delivery. The phase transition temperature (PT) of lipids, at which the lipid molecules change from liquid crystal to gel phase, significantly influences the fluidity, stability, molecular encapsulation, and release properties of liposome membranes. Phospholipids typically exhibit multiple PTs, enabling transitions into various morphologies (such as crystalline, gel, liquid-ordered, and liquid-disordered), each with distinct characteristics [37]. Therefore, the stability, transportation efficiency, and release characteristics of liposomes can be precisely controlled by choosing appropriate lipids and adequate PT to enhance liposomes for in vivo applications.

Liposome preparation technology

The conventional approach for liposome preparation is the thin-film hydration technique introduced by Bangham in 1964 [38]. Phospholipids are solubilized in an organic solvent (such as chloroform), and the solvent is subsequently evaporated to create a thin membrane, and the membrane is fused to a hydration medium. In this process, the drug can be contained in an aqueous medium or in a lipid membrane, which is hydrated to rearrange the membrane into a bilayer structure, forming liposomes (Fig. 2). However, this method has a low rate of hydrophilic drug encapsulation (5–15%).

Solvent injection [39] and reverse evaporation [40] optimize the principle of inducing the self-assembly of phospholipids by rapid solvent mixing or emulsification in the aqueous phase, they feature a significant reduction in the impact of organic solvents on phospholipids as a way to promote encapsulation efficiency (50%–80% and 60%–90%, respectively). To guarantee the dimensions, lamellarity, and homogeneity of liposomes, post-molding treatment is essential, and ultrasonication, high-pressure homogenization, and supercritical fluid methods play a role in this process. Ultrasonication [41] uses high-frequency ultrasound's mechanical vibration effect to modulate liposome size at the physical level; high-pressure homogenization [42] uses the high-speed jet of liquid when the phospholipid solution passes through a narrow channel to disperse and homogenize liposomes, which is capable of minimizing liposome size; the supercritical fluid approach [43] uses non-toxic supercritical fluids like carbon dioxide to co-solubilize phospholipids with the medication and produce liposomes

Table 1 Scheme of liposomes classification. (Created with BioRender.com) [20–32]

Vesicle type	Size	Layers	Structural diagram	Application in cancer
SUVs	20-100 nm	Single		Imaging[20,21] Eradication[22]
LUVs	100-1000 nm	Single		Drug delivery[23]
GUVs	>1000 nm	Single		Tumor imaging[24] Detection[25]
OLVs	100-1000 nm	Multi-nonconcentric		Drug delivery[26] Treatment[27]
LMVs	>500 nm	Multi-concentric		Drug delivery[28,29] Treatment[30]
Multi-vesicular vesicles	>1000 nm	Multi-nonconcentric		Drug delivery[31] Drug stable and enhance bioavailability[32]

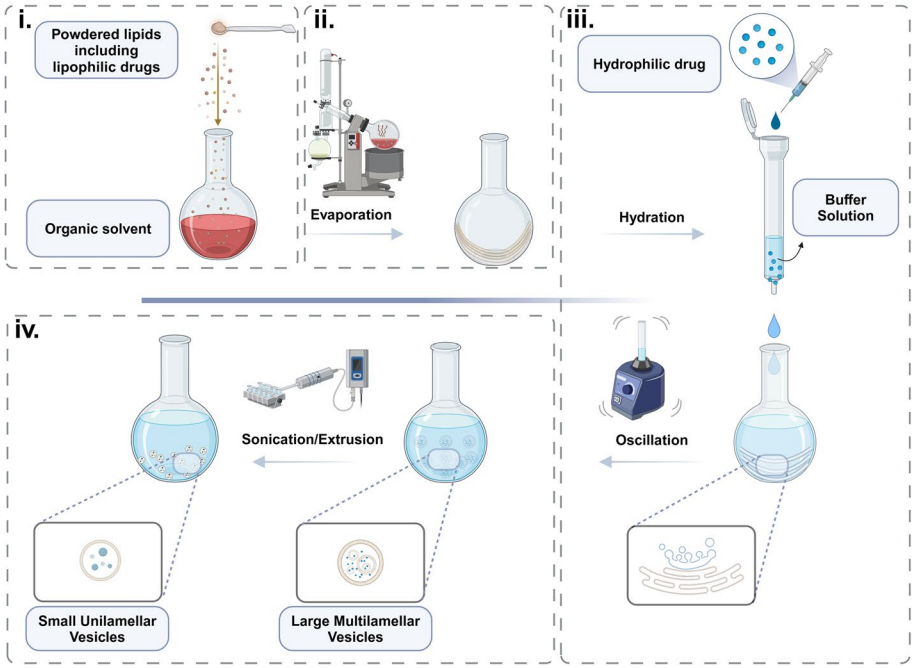


Fig. 2 Schematic diagram of liposome preparation by conventional method. **i** Solubilize lipids in a chloroform/methanol solvent system. **i**) Evaporate the solvent to create a thin lipid film using a rotary evaporator under reduced pressure. **iii** Hydrated films, in conjunction with buffers (such as PBS), undergo gentle oscillations at 37 °C to generate enormous multilamellar vesicles. **iv** Homogenize liposomes to generate unilamellar vesicles using sonication or extrusion through a polycarbonate membrane. (Created with BioRender.com)

by modifying pressure and temperature. The fluid can be rapidly metabolized and eliminated in the body, which can reduce the biotoxicity and immune response to the body, and this fusion-forming mode effectively enhances the biocompatibility of liposomes.

These approaches can dynamically tune ULV and MLV synthesis to enable liposome-based functional applications (ULV for efficient cellular absorption and quick drug distribution, MLV for slow release and macromolecular delivery). In addition, alternative and novel preparation methods such as curvature modulation, osmotic impaction, and double asymmetric centrifugation can be learned from the review of Has et al. [44]. These new liposome production methods have stabilized their structures and characteristics and boosted production and encapsulation efficiency. Modified liposomes have a wider range of applications in the molecular field, in which liposome technology for tumor-targeted biomarkers focuses on molecular recognition and cancer detection.

Liposomes in CTCs

CTCs were first identified in 1869 by Ashworth in the peripheral blood of cancer patients [45]. CTCs are either single cells or clusters related to cancers, with the latter being linked to a higher metastatic potential, enabling them to travel through blood circulation to distant organs and infiltrate the mesenchyme of distant tissues [46]. As indicators of tumor burden, CTCs provide important genomic, transcriptomic, and proteomic information that reflects cancer progression. However, their rarity (~ 1 CTC/10^7 blood cells) poses a significant challenge for detection and analysis [47]. To overcome this, precise capture and sensitive detection methods are essential. Liposome-based nanocarriers offer a promising solution due to their versatility and fluidity, which allow customize nanostructures for efficient CTC capture and detection. This section focuses on the application of liposomes for the specific capture and detection of CTCs.

Liposome-based CTCs capture

Targeted recognition of CTCs is achieved by binding specific molecular probes or ligands to specific antigens or markers on the surface of CTCs [48], to achieve selective recognition and enrichment of CTCs and analysis of them further to learn about information such as tumor metastasis, gene mutation profiles, and their response to treatment is significant in comprehensive CTC researches. Current CTC capture methods primarily cover physical property-based techniques, including size difference and density separation [49, 50], as well as

biological property-based techniques, such as antibody-labeled immunocapture [51, 52]. Nevertheless, these techniques still have drawbacks including significant false positives and membrane-damaging effects.

To tackle the inherent challenge of isolating CTC from peripheral blood, liposome-based probes emerged. For instance, Kuai et al. [53] designed epidermal growth factor receptor (EGFR)-targeted immune magnetic liposomes (EILs) for targeting and efficiently capturing colorectal CTCs. They dissolved the modified DSPE-PEG-EGFR, added bare magnetic nanobeads to obtained deformable antibody receptor-lipid bilayer structures EILs. Utilizing improved interactions between EILs and nanoscale cell surface components, tricolor immunocytochemistry was used to identify and quantify CTCs in non-specifically captured leukocytes. At the criterion of 7.5 mL of total blood volume, the number of CTCs that are captured by EILs (5–181 CTCs) was substantially higher compared to the traditional method of EpCAM magnetic beads (15–79 CTCs), and it has significant diagnostic value for KRAS gene mutations in colorectal cancer CTCs [53]. Liposomes in this process integrate a high-affinity cell enrichment analysis system and a cell membrane structure that enhances the frequency of CTC and substrate interactions. In addition, encapsulated probes can be protected for longer internal circulation, thereby improving the contact rate between EIL structures and cancer cells, which enhances the efficiency of CTC capture (Fig. 3A). Furthermore, Kang et al. [54] presented a method for the integrated capture and detection of CTCs utilizing bioorthogonal click chemistry in conjunction with hybrid EVs-liposomes camouflaged magnetic vesicles. The capture phase of this method facilitates the efficient isolation of CTCs through the use of a liposome-EV-camouflaged magnetic vesicle composed of dibenzocyclooctyne (Fe3O4@lip/ev-DBCO). This vesicle is capable of undergoing a bioorthogonal click chemistry reaction with azide groups that are produced via metabolic labeling on the surface of tumor cells. This approach has an extremely low limit of detection (LOD) of 10 CTCs. The adsorption performance was outstanding, with an average of 7.1 ± 3.6 CTCs extracted from experimental samples (blood of mice with melanoma lung metastases) (Fig. 3B).

However, these strategies both have innegligible issue that the false positive capture of normal cells. Although

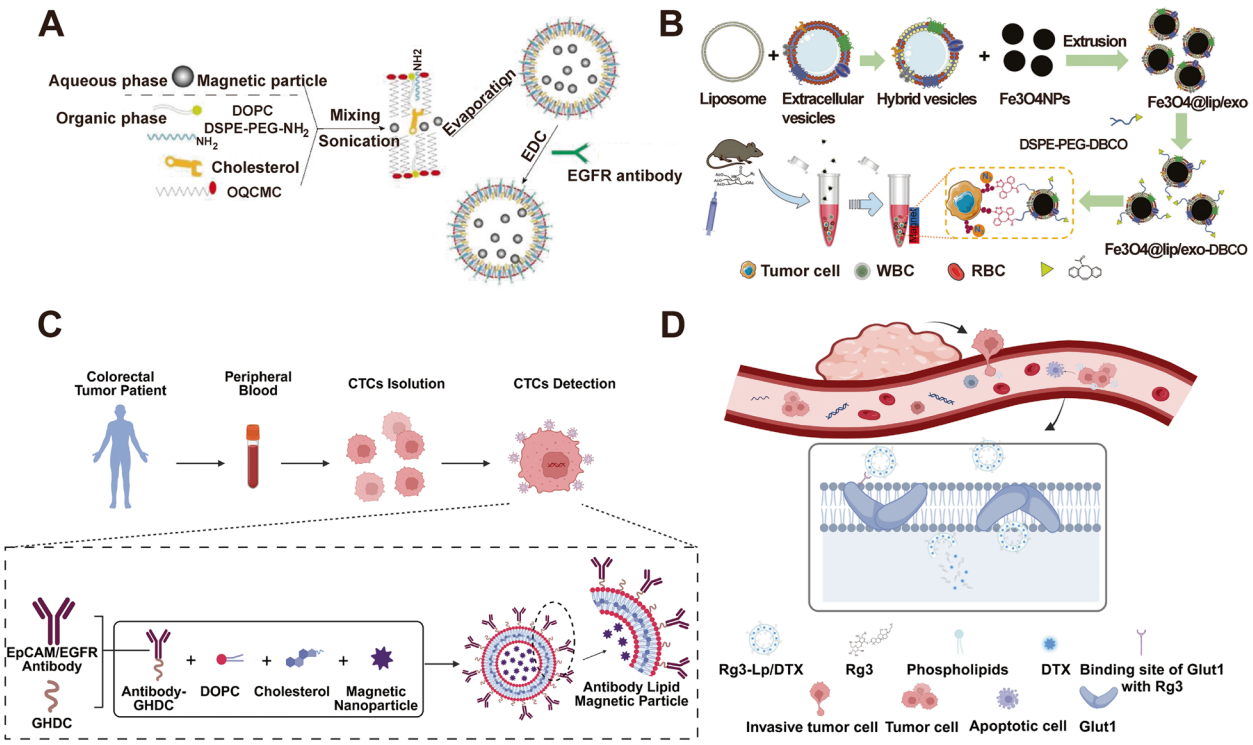


Fig. 3 Liposome-based capture in CTCs. **A** Schematic illustration of the preparation of EGFR-targeted immune magnetic liposomes [53]. Reproduced with permission. Copyright © 2018 Baishideng Publishing Group Inc. Licensed under CC BY-NC 4.0. **B** Schematic illustration of the CTCs enrichment by EVs-camouflaged magnetic vesicles cooperating with bioorthogonal click chemistry [54]. Reproduced with permission. Copyright © 2023 John Wiley & Sons. **C** Schematic diagram of IMMSs preparation and CTCs detection. Inspired by Chen et al. [55]. **D** Schematic illustration of Rg3-Lp/DTX preparation and CTC detection. Inspired by Xia et al. [56]

both of them use tricolor immunofluorescence to limit interference from normal cells, the actual capture specificity is limited. In this context, it may be advantageous to do a dual screening for tumor markers (such as FAS) post-capture or to create tumor microenvironment-activated metabolic precursors (such as pH-sensitive derivatives of Ac4ManNAz) to reduce azide labeling in normal cells.

Liposome-based CTCs detection

Cancer biogenesis, recurrence, and metastasis are the primary factors contributing to the short survival of patients [57]. As malignancies progress, the quantity of CTCs in the body progressively rises [58], the enumeration of CTCs and the measurement of tumor-derived EV concentration directly reflect the cancer state [59], which offering a novel insight into tumor diagnosis, prognosis, and therapy assessment. Existing CTC detection methods mainly include fluorescent staining techniques and flow cytometry sorting [60], but the LOD values of these techniques are generally low and cannot be applied to early and precise detection of cancer. Liposomal assay technology therefore can be a hoping way to achieve more accurate and specialized detection of CTC (Table 2).

Currently, several targeted technologies are available for CTC detection, each offering unique advantages and characteristics. Chen et al. [55], for example, developed a method to construct multi-site liposomes of immunomagnetic microspheres (IMMS) for identifying CTCs in non-small cell lung cancer (NSCLC) patients' blood. They used liposomes to encapsulate Fe_3O_4 nanoparticles and modified surface EGFR antibodies to form EGFR/EpCAM multisite IMMS and accurately recognize CTCs. This method has a very high positive sorting rate, with more than 90%. In contrast to standard antigen–antibody reaction-based detection approaches, the team's methodology integrates several recognition sites to evaluate the target in many dimensions, preventing false detection owing to genetic modifications and mutations (Fig. 3C). However, the static design of marker combinations is ineffective for monitoring tumor dynamics. A "modular magnetic bead-liposome platform" may be constructed to provide liposome carriers using interchangeable antibody modules, allowing for dynamic adjustments to the target combination in response to alterations in CTC phenotype during patient treatment, hence enhancing detection efficiency.

It's worth mentioning that Xia et al. [56] already developed a multifunctional ginsenoside Rg3-based liposome loaded with docetaxel (Rg3-Lp/DTX), achieving accurate capturing of CTCs by a distinctive technique. Rg3 functions as a molecular recognition cofactor that can substitute for cholesterol, enhancing the stability and fluidity of liposome membranes. The steroidal ring structure of Rg3, the C17 side chains and the hydroxyl group at the C3 site in this vector fulfill the conditions for use as a liposomal membrane modulator, which inserts into the liposome membrane to interact with the phospholipid bilayer and extends its specialized glucose moiety to the liposome surface, allowing it to trap CTC in body fluids more efficiently by interacting with Glut1. In experiments replicating the CTC microenvironment, the capture efficiency of Rg3-Lp to simulated cells that was 2.17 times greater than that of typical cholesterol liposomes. This modified liposome platform achieves specific targets accurate identification (Fig. 3D).

Liposomes in EVs

EVs are membrane vesicles secreted by cells, including tumor cells. Based on their size, origin, and biological properties, EVs are classified into microvesicles, exosomes, ectosomes, and oncosomes [66]. EVs play a crucial role in intercellular communication by transferring biomolecules (such as proteins and miRNAs) to target cells, regulating tumor processes, and influencing cell function via autocrine and paracrine signaling [67–69]. Due to their tumor-specific molecular content, EVs are considered potential biomarkers, reflecting tumor biology. However, challenges remain in using EVs for tumor detection, including their heterogeneity, low

Table 2 Liposome-based CTCs detection

Method	Sample	Capture rate	Reaction time	References
Immunomagnetic liposome	Serum of NSCLC patients	90%	—	[55]
EVs-liposome camouflaging	Blood of melanoma-bearing mice	80%	40 min	[54]
Multimarker magnetic liposome	Blood of multi-tumor patients	80%–97.5%	35 min	[61]
Immunomagnetic liposome	Cerebrospinal Fluid and blood of brain tumors patients	92.4%	25–30 min	[62]
Amphiphilic liposome	Blood of multi-tumor patients	62.82%–89.09%	—	[63]
Liposome-tethered supported lipid bilayer	Cell and cancer patients' blood	70%–78%	—	[64]
Magnetic capture column	Blood infused with cancer cells	80.7%	11 min	[65]

concentration in body fluids, and membrane fusion properties, which complicate the capture and identification of EV subpopulations [70]. To address these issues, liposomes are increasingly used in EV-related studies due to their efficient trapping ability, biocompatibility, and ease of functionalization. This section will review the application of liposomes in the capture and detection of EVs.

Liposome-based EVs capture

The swift and accurate isolation of EVs from bodily fluids is crucial for illness diagnosis and clinical assessment [71]. Currently, several different separation techniques for EVs have been developed based on their physicochemical and biological functions, including ultracentrifugation, transmission electron microscope, nanoparticle tracking analysis, and density gradient separation [72–74]. They utilize different principles and traits for efficient isolation of EVs. In addition, liposome-based techniques have been constructed for the capture and separation of EVs (Table 3).

How to selectively capture heterogeneous EVs with simple and reliable tools and platforms is one of the main hotspots for research targeting EVs nowadays. Kim et al. [75] invented a polydiacetylene (PDA) liposome immunosensor for the exosomes' detection, and they constructed exosome-specific liposome probes by attaching anti-CD63 monoclonal antibodies to PDA liposomes. Due to the unique optical properties of PDA [81], tetratransmembrane proteins CD63 on the surface of exosomes undergo antigen–antibody interactions with antibodies coupled to PDA liposomes when exosomes are present. In this phase, antioxidants may be included into PDA membranes to reduce the photobleaching effect [82]. This interaction changes the structure of the conjugated backbone of PDA, which induces a colorimetric change (from blue to red) and an alteration of the fluorescence properties of PDA liposomes, by which accomplish the identification and quantitative analysis of exosomes. The LOD is 3×10^8 vesicles/mL. The liquid-phase sensor in this method eliminates the need for additional functional chips and devices and has great potential for the capture of EVs from malignant tumors (Fig. 4A). In addition to being used as probes for direct EV capture, liposomes also have the ability to indirectly detect EVs. Xu et al. [76] described a technique for the quantification of sEVs with size exclusion chromatography with fluorescence detection (SEC-FD). They utilized lipophilic CM-Dil dye for the fluorescent labeling of sEVs and monitored the fluorescence of the eluate. In this method, liposomes do not directly identify EVs or their markers but are used to distinguish the distinct chromatographic behavior produced by small EVs relative to the background. This method's detection range is from 2.0×10^8 to 1.5×10^9 sEVs particles/mL, and the LOD is 2.9×10^7 particles/mL. This approach allows for the analysis of sEVs without enrichment, enhancing the convenience of EV detection in malignant tumors (Fig. 4C).

Liposome-based EVs detection

EVs are composed of proteins and nucleic acids (miRNAs are vital nucleic acid markers) [83, 84], which reflect the phenotype of their parental cells. Therefore, tumor-derived EVs contain miRNAs and proteins that can be used to localize and identify tumor information and serve as significant indicators in malignancy biopsies.

Detection of RNA of EVs

RNAs are a class of short, coding (some of them non-coding), single-stranded fragments that can play an important role in gene expression regulation by inhibiting the translation of target genes or degrading the corresponding transcribed material [85]. Tumor-derived EVs encapsulate a variety of RNAs and circulate throughout the body, which are closely associated with tumor progression and metastasis [86, 87]. The current common methods of EV RNA detection include RT-qPCR [88], microfluidics [89], and nuclease-assisted signal amplification [90], which have long detection times and possible

Table 3 Liposome-based EVs capture

Methods based EVs capture	Sample	Technique	LOD (particles/mL)	References
Liposome-immunosensor	Plasma vesicle solution	Immunofluorescence	3×10^8	[75]
Liposome-SEC-FD	TK6 cells	Chemical fluorescence	2.9×10^7	[76]
Liposome-coordination chemical bridging	HeLa cell	Immunofluorescence	7.6×10^6	[77]
Multitarget liposome-biosensor	Human plasma	Immunofluorescence	4×10^7	[78]
Liposome-Hybridization chain reaction	Serum of fetal bovine and cancer patient	Chemical fluorescence	2.2×10^6	[79]
Paper-based lipid bilayer biosensor	Human serum	Chemical fluorescence	5×10^{-3}	[80]

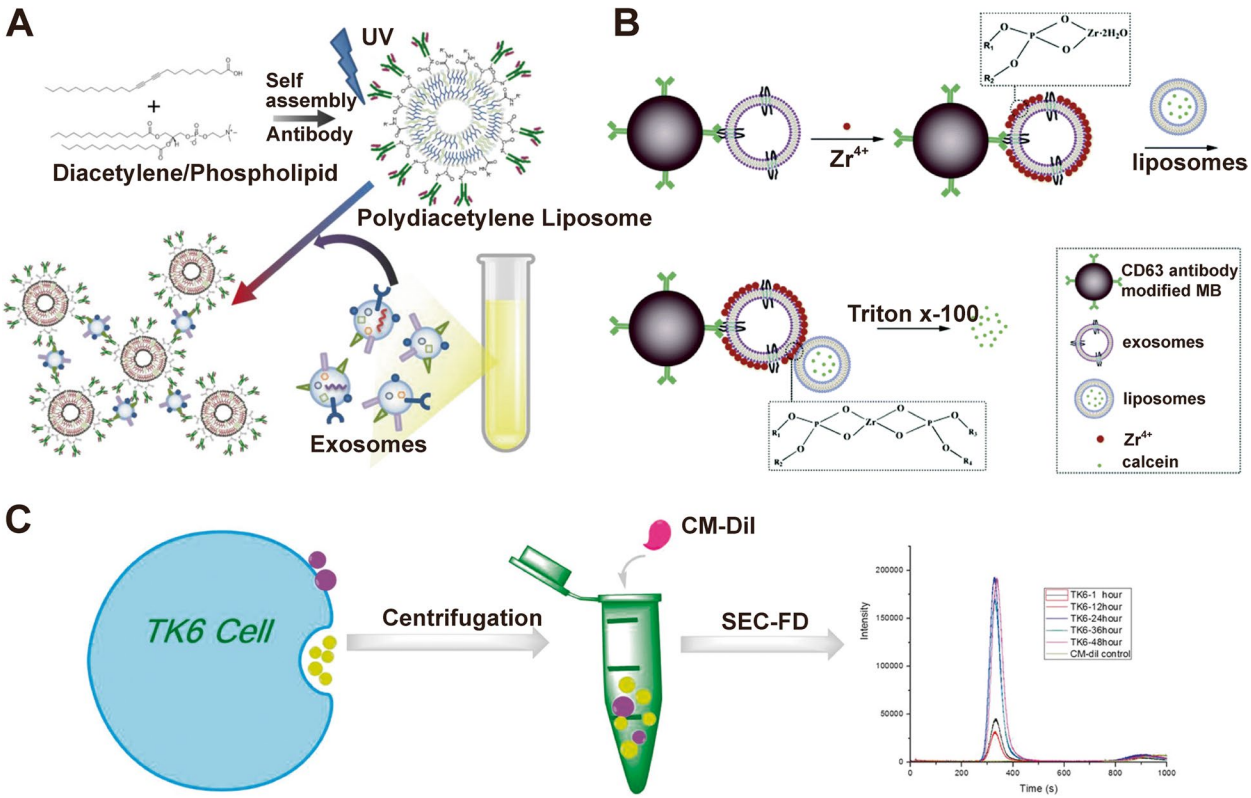


Fig. 4 Liposome-based capture in EVs. **A** Schematic illustration of using liposome immunofluorescence capture exosome [75]. Reproduced with permission. Copyright © 2019 American Chemical Society. This publication is licensed under CC-BY-NC-ND. **B** Schematic illustration of using liposome-based coordination chemical bridging capture EVs [77]. Reproduced with permission. Copyright © The Royal Society of Chemistry 2019. **C** Schematic illustration of the distinction principle of SEC-FD chromatograms [76]. Reproduced with permission. Copyright © 2016 American Chemical Society

contamination. The application of these techniques to accurately distinguish and quantitatively detect heterogeneous EVs and different RNAs under complex background interference remains challenging [91]. In contrast, liposomes serve as high-capability tool for EVs RNA detection.

Zhang et al. [92] designed a liposome technology utilizing a combination with gene editing technology to directly detect plasma miRNAs by a liposome-mediated membrane fusion strategy-transfected CRISPR/Cas13a (MFS-CRISPR). This platform was able to be applied to the analysis of heterogeneous exosomes, and it was able to distinguish the difference in miR-21 expression between breast cancer patients and healthy donors ($P < 0.05$) with an AUC of 0.84. It can detect exosomal miR-21 over a linear range of four orders of magnitude (10^4–10^8 particles/mL) and has a LOD as low as 1.2×10^3 particles/mL, which is at least 1000-fold more sensitive than conventional MB strategies (Fig. 5A). In addition, in situ detection of EV miRNAs is also a topic of significant importance in the current environment. Lei et al. [93] designed a unique method for in situ detection of miRNAs, which anchors a complementary DNA tag of specific tumor and exosome markers to the liposome probe. It then hybridizes in a zipper-like behavior with orthogonal identity barcodes guided by dual surface proteins (CD63 and EpCAM) on EVs' surface, by which accurately identifying tumor-derived EVs. Afterward, Au NFs and double-strand-specific nucleases in liposomes were used to generate and amplify the fluorescent signals and enable in situ miRNA detection. This method is capable of functioning in 100-fold dilutions of serum and has 100% sensitivity, specificity, and accuracy in distinguishing prostate cancer from benign prostatic hyperplasia among a combination of six clinically validated miRNA markers, which has potential miRNA molecular phenotypic adaptations and good cancer diagnostic performance (Fig. 5B). Its main advantage is the ability to eliminate the interference of irrelevant free miRNAs and the extremely low background and can provide amplified fluorescence signals, co-working to achieve accurate in situ diagnosis of tumor miRNAs.

It is important to acknowledge that in EV-RNA detection, neither method eliminates the interference of

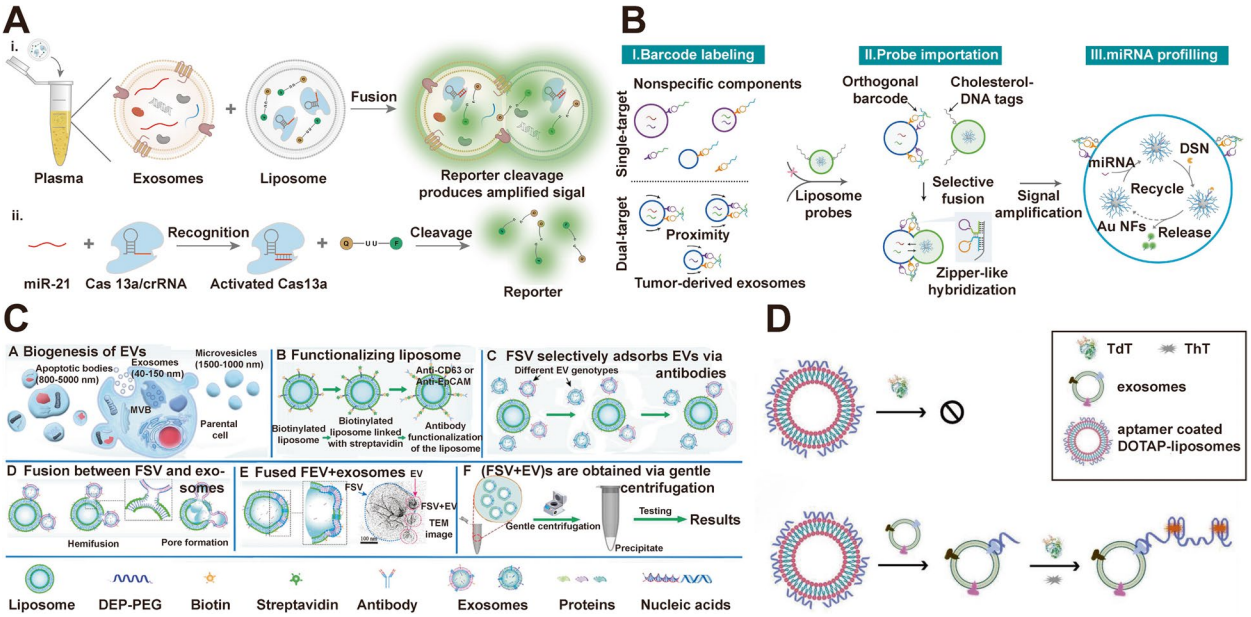


Fig. 5 Liposome-based detection in miRNA and proteins in EVs. **A** Schematic illustration of MFS-CRISPR platform detection of exosomal miRNAs [92]. Reproduced with permission. Copyright © 2023 American Chemical Society. **B** Schematic illustration of dual-surface-protein-guided orthogonal recognition barcode for detecting exosomal miRNAs [93]. Reproduced with permission. Copyright © 2023 American Association for the Advancement of Science. Licensed under CC BY-NC 4.0. **C** Schematic illustration of preparation of FSV used in detecting exosomal proteins [94]. Reproduced with permission. Copyright © 2023 American Chemical Society. **D** Schematic illustration of DOTAP liposomes pooling suitable ligands to capture exosomes [95]. Reproduced with permission. Copyright © 2019 American Chemical Society

non-specific background signals. For this issue, the precision and consistency of detection can be further enhanced by using internal reference signals, while the impact of matrix effects and operational variability can be reduced [96].

Detection of proteins of EVs

Proteins in EV can be categorized into membrane proteins, cytoplasmic proteins, nuclear proteins, and functional enzymes, which are important functional components of EV. They can also play an important role in tumorigenesis and metastasis by assisting in the construction of the tumor microenvironment, mediating tumor metastasis, and transmitting metabolic signals [97, 98]. Currently, the main conventional methods for detecting EV proteins are ELISA [99], Western blot [100], mass spectrometry (MS) [101], and molecular imaging [102], but they rely on specific antibodies and high-specification instruments. Liposomes have been used as a new way to detect EV proteins; they have membrane fusion and surface modification properties that allow them to bind proteins rapidly, and they have the advantages of being more sensitive, stable, non-destructive, and multiplexable compared to traditional methods [9].

Based on the binding of liposomes to functional antibodies against EV proteins, Cheng et al. [94] designed an antibody-functionalized synthetic vesicle body (FSV) strategy capable of capturing EVs in dermal interstitial fluid. This method has a high EV saturation point of 1.8 $\times 10^{11}$ particles/mL, and the efficiency of EV detection was 100% when the concentration was below this saturation point. This FSV strategy also captured EV proteins much more efficiently than the ultracentrifugation method (Fig. 5C). Importantly, this method presents challenges in controlling the direction and density of the conjugated antibody on the liposome, potentially hiding the Fab region of the antibody's active binding site and consequently diminishing the binding efficacy of EpCAM/CD63. This issue can be addressed by pre-modifying liposomes with conjugated receptors to guarantee that the Fab portion of the antibody is oriented towards the solution [103]. The combination of EV testing with aptamers has also garnered significant attention [104]. Wang et al. [95] described a terminal deoxynucleotidyl transferase (TdT)-based nucleic acid aptamer-liposome complex for capturing exosomes. They use electrostatic interaction to cause positively charged DOTAP liposomes to adsorb nucleic acid aptamers, and this binding creates a spatial site barrier that prevents TdT from recognizing the aptamer or allowing it to extend. When exosomes are present, this property enables the aptamer to bind to the EVs-specific surface membrane protein

CD63 (which is present only on the surface of exosome membranes), and TdT recognizes the 3′-OH end of the aptamer and forms multiple G-quadruplex structures in a dGTP-rich environment, which binds to thioflavin T to produce strong fluorescence. This LOD of the method is 3.6×10^5 cells/mL. The key to this approach is to achieve enzymatic signal amplification by capturing specific proteins to enable the extension of the nucleic acid aptamer chain based on TdT modifications (Fig. 5D). The above method not only addresses the current gap of simple and reliable tools to selectively capture heterogeneous EVs and non-destructively convey internal protein information in EV protein mapping and spatial proteomics studies but also adds a new guiding scheme for the identification of EVs of malignant tumor origin.

Liposomes in CTPs

CTPs are specific proteins secreted by tumor cells into the circulation, including cytokines, enzymes, and signaling molecules [105]. These proteins are released during apoptosis, lysis, or necrosis, and indirectly regulated through the remodeling of the tumor microenvironment [106]. CTPs are key indicators of tumor type, stage, aggressiveness, and therapeutic response, reflecting the dynamic activity and metabolic state of tumor cells. As important tumor biomarkers, CTPs provide valuable insights into cancer progression. [107]. However, current detection methods, including ELISA [108], MS [109], protein microarray [110], and surface plasmon resonance [111], suffer from low sensitivity, limited marker detection, and insufficient dynamic range, hindering their clinical applicability. Liposomes offer a promising solution for CTP detection due to their membrane affinity, large surface area, and functionalized modification characteristics.

Liposome-based protein markers detection

Tumor proteins, such as AFP and CES, are closely associated with cancers like hepatocellular carcinoma and colon cancer, where they are highly expressed [112, 113]. These proteins are typically detected through antigen–antibody interactions. In contrast, liposomes possess numerous ligand-binding sites on their surface, the ligands can be antibodies, small molecules, and synthetic ligands like folic acid and peptides [114]. These sites can accommodate multiple probe molecules, enabling efficient 1:n signal amplification and delivery.

Liposomal probes enable ultrasensitive detection of cancer protein molecules. For instance, Liu et al. [115] developed a biotinylated liposome-based immuno-loop-mediated isothermal amplification (LI-LAMP) approach for urine cancer protein marker identification. The assay system utilizes a sandwich ELISA framework. Initially, dsDNA is encapsulated in single-shell liposomes as amplification substrate. Subsequently, a streptavidin–biotin bridge links biotin-labeled liposomes to the REG1A target in the reaction system, generating an immunosandwich structure. And finally, the released dsDNA amplified by LAMP system. As amplification proceeds, fluorescent signals can be detected, and protein concentration can be quantified by a concentration dependence curve of Ct values versus REG1A (Fig. 6A). This method's primary advantage lies in its ability to prevent static or collisional bursting of fluorescent molecules by the lipid bilayer. Besides, the biocompatible environment offered by the liposomal probe enhances resistance to nonspecific adsorption in serum backgrounds. Subsequently, Liu's team [116] reported a magnetic-bioluminescent-nanoliposome (LBM) technique for protein markers' immediate detection. The team encapsulated reporter adenosine 5'-triphosphate disodium salt hydrate (ATP) within nanoliposomes and conjugated ATP-encapsulated nanoliposomes with anti-AFP rabbit polyclonal antibodies using the glutaraldehyde coupling method, functioning as a signal detection probe. The amidation reaction was employed to couple carboxylated magnetic beads with anti-AFP mouse monoclonal antibodies, serving as a solid capture carrier. The structure guided the formation of magnetic nanoparticles-AFP-liposome nanocomplexes through a sandwich reaction involving the target protein. The key to this approach is the use of ATP to mediate the bioluminescence effect and the transmission of fluorescent signals [117]. By attaching numerous ATP molecules around a target, enzymatic reaction cascades can double luminescence and fluorescence signals. The method has very high sensitivity and marker specificity, with a LOD of 0.016 ng/mL for AFP concentration and a linear range of 0.05 to 1000 ng/mL (Fig. 6B). It is a major advance over standard ELISA (3.4 ng/mL) and localized surface plasmon resonance (LSPR) (24 ng/mL) techniques, enabling early hepatocellular carcinoma identification with multisite binding.

Liu's two research have successfully accomplished the portable and sensitive detection of low-concentration protein markers. Nevertheless, the controllability of signal release, including variations due to liposome membrane leakage and inadequate cracking efficiency, has not received adequate attention. In the subsequent trials, liposome production parameters may be refined, and innovative lysis techniques may be employed to enhance the outcomes.

Liposome-based tumor-specific enzyme detection

Enzymes are protein or RNA molecules produced by living cells in living organisms that have catalytic functions. The activity or expression levels of certain tumor-specific

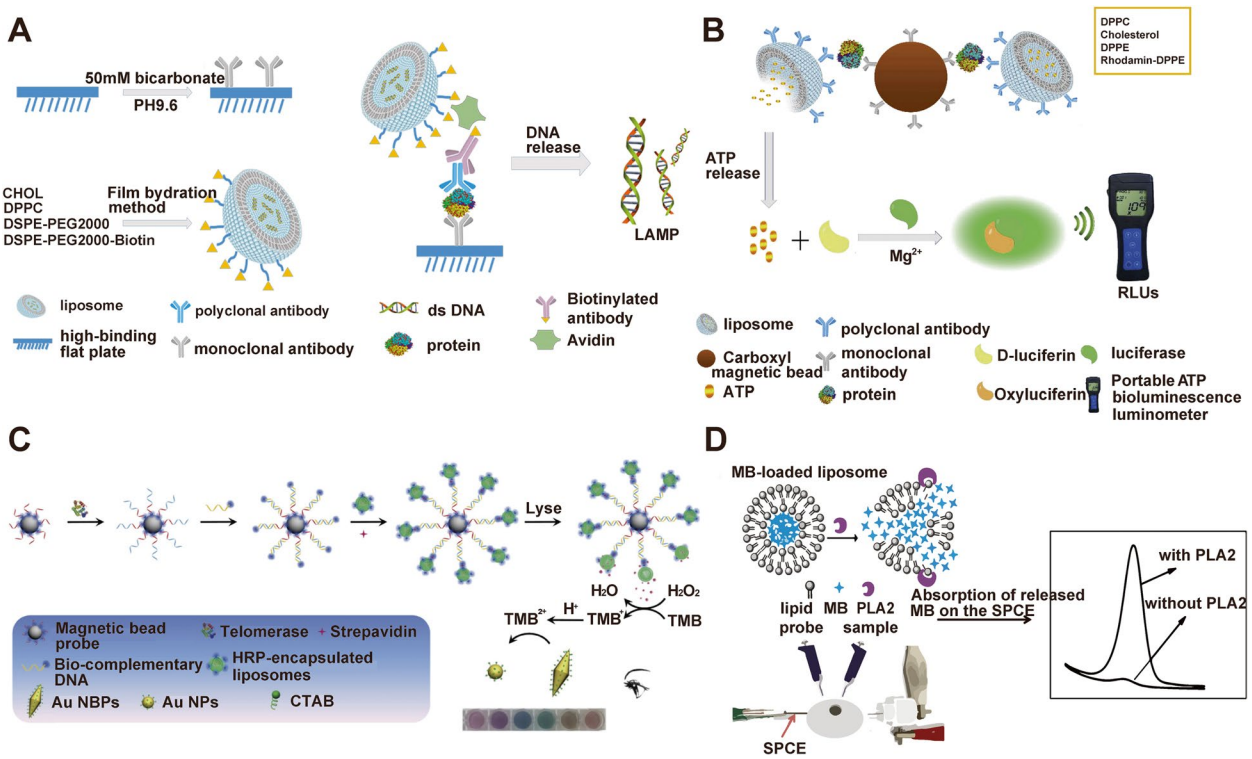


Fig. 6 Liposome-based detection of CTPs. **A** Schematic illustration of the LI-LAMP assay for REG1 A in urine [115]. Reproduced with permission. Copyright © 2017 Elsevier B.V. **B** Schematic illustration of the LBM assay for portable and quick detection of proteins [116]. Reproduced with permission. Copyright © 2018 Elsevier B.V. **C** Schematic illustration of the etching-based plasmonic colorimetry for telomerase activity detection [118]. Reproduced with permission. Copyright © 2019 Elsevier B.V. **D** Schematic illustration of the release of PLA2 and detection of electrical signals [119]. Reproduced with permission. Copyright © 2018 Elsevier B.V

enzymes are significantly abnormal in tumor tissue, for example, matrix metalloproteinases are overexpressed in cancer cells [120]; lactate dehydrogenase activity is increased in tumor cell metabolism, and measuring its level can indicate the metabolic status of the tumor [121]. Thus, enzymes can be employed not only to catalyze chemical pathways, but also as biomarkers for tumor identification. Liposomes' targeting, controlled release, and biocompatibility make them ideal for detecting tumor-specific enzymes.

Serum phospholipase A2 (PLA2) levels substantially correlate with various cancers [122]. Based on this, Zhang et al. [119] designed a liposomal probe to accurately diagnose malignancies. The chosen method enriches PLA2 signaling molecules directly on a 3D graphene-like screen-printed carbon electrode (SPCE) for electrochemical measurement. This method employs PLA2 to enzymatically cleave and release methylene blue (MB) attached to the liposomal probe. Subsequently, the 3D graphene-like structure enhances MB uptake to the surface via π-π interactions. Finally, highly sensitive and selective PLA2 activity detection can be achieved by detecting the oxidation current of adsorbed MB. The current intensity was linearly proportional to PLA 2 activity from 5 to 200 U/L, with a LOD of 3 U/L (Fig. 6D). This method innovatively employs the multilayer structure of graphene to enhance active sites and amplify signals, thereby improving detection sensitivity. Nevertheless, aromatic metabolites, including tryptophan and uric acid, present in serum can engage in competition with MB for liposomal probe binding sites via π-π interactions, leading to a suppression of the signal [123]. Consequently, it is essential to modify the surface characteristics of graphene to diminish the non-specific adsorption of liposome probes to negatively charged interferents.

Furthermore, the detection of enzymes is further enhanced by the addition of functionalized magnetic liposomal particles to electrochemistry. Wang et al. [118] investigated a plasmonic colorimetric biosensor utilizing horseradish peroxidase-encapsulated liposomes (HRP-Ls) for the detection of tumor-specific telomerase activity. The telomerase substrate primer elongates with telomerase to produce particular sequences that hybridize to the cDNA. The catalytic reaction after HRP release produces TMB^{2+} etching Au NBPs, which causes

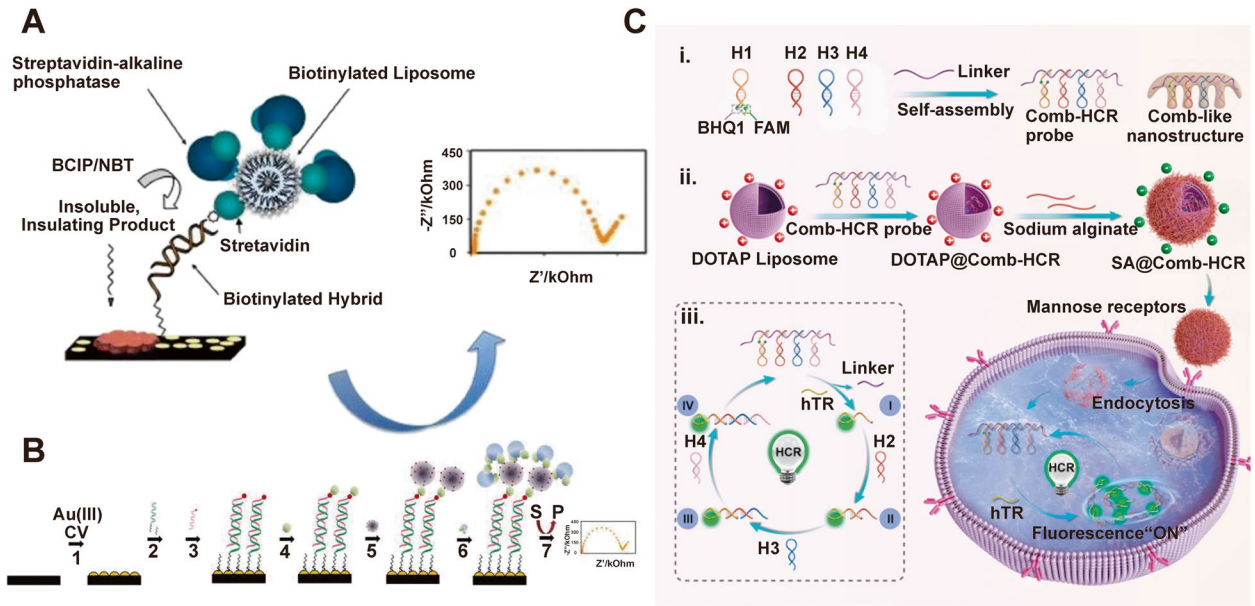


Fig. 7 Liposome-based detection of ctNAs. **A** Schematic illustration of the structure principle of tumor-associated RNA detection [130]. Reproduced with permission. Copyright © 2016, Springer-Verlag Berlin Heidelberg. **B** Schematic illustration of the assay of using EIS [130]. Reproduced with permission. Copyright © 2016, Springer-Verlag Berlin Heidelberg. **C** Schematic illustration of SA@Comb-HCR for hTR detection [131]. Reproduced with permission. Copyright © 2024 Elsevier B.V

LSPR peaks blue-shifts and color changes. HRP-Ls then amplify the cascade signal and can be used to quantify telomerase activity by visual observation or LSPR band shifts (Fig. 6C). This method demonstrates a visual LOD of 20 HeLa cells (extracted telomerase), with the LSPR peak displacement (Δλ) showing a positive correlation to the number of HeLa cells within the range of 5 to 1000 cells, with LOD being 1 cell. This technology offers novel avenues for visual and quantitative assessment of tumor telomerase activity, showing significant potential for enzyme detection. Hence, the utilization of liposomes for the direct detection of malignancy-associated enzymes illustrates the viability of employing liposomes to identify tumor-specific enzymes.

Liposomes in ctNA

ctNAs are a class of nucleic acids derived from tumor cells. Physiological processes including the release of secretions, lysis, and necrosis of tumor cells, result in their release into the body's peripheral blood [124]. The main ctNAs that have been observed to be significantly increased in cancer patients blood include cell-free DNA (cfDNA), messenger RNA (mRNA) and non-coding RNA (ncRNA) [125–127]. Due to the heterogeneity of different types of ctNAs, they vary in abundance and specificity in the organism, which represent the heterogeneity, genetic traits, and dynamics of the originating tumor cells [128, 129]. Thus, they may function as significant entity for tumor detection, personalized therapy, and tumor-load assessment. However, the dependence on coding complementarity and the diminutive size following their release into the circulatory system present a challenge for subsequent detection. Liposome has become an effective approach for ctNA binding and detection in vivo.

Voccia et al. [130] successfully detected miR-222, a molecule closely associated with tumorigenesis, by electrochemical impedance spectroscopy (EIS). They used enzyme-decorated liposomes and nanostructured screen-printed electrodes to improve the impedance method (Fig. 7A). The insulating liposomes on the electrode surface interrupt the interfacial electron transfer process, leading to an increased electron transfer resistance (R_{et}) value. The precipitation of insoluble products resulting from the hybridization and biocatalytic activity of the enzyme enhances the insulating layer R_{et} on the electrode, leading to the amplified detection of miR-222 signals. The method's LOD is 0.400 pM, the quantification limit is 1.70 pM, which range spans three orders of magnitude (Fig. 7B). There is also no cross-reactivity with non-complementary sequences. As a complement to the enzyme assays described above, Chen et al. [131] employed the SA@Comb-HCR nanosystem for the detection of hepatocellular carcinoma-associated telomerase RNA (hTR). Sodium alginate (SA)-modified DOTAP liposomes were utilized to encapsulate comb-HCR probes. SA interacts with the mannose receptor

(MR) on HCC cell membranes through receptor-ligand interactions, promoting the aggregation of the nanosystems at the tumor site for the delivery of probes and detection of markers. The nanoprobe is highly specific and sensitive for hTR detection with LOD as low as 0.7 pM (Fig. 7C). Liposomal nucleic acid detection techniques possess established detection principles like sandwich hybridization assays and electrochemical analysis, by which they are widely applied in identifying various marker nucleic acids, including viral and cellular types [132–136]. Nonetheless, the research application concerning the capture and detection of tumor-specific ctNAs remains underdeveloped. To efficiently capture and analyze tumor-associated gene mutations in blood and accurately assess the genetic characteristics and dynamics of tumors, the continuous development of liposomal platforms for direct detection of tumor nucleic acids is essential.

Conclusion and outlook

Liposomes augment conventional tumor marker detection approaches in marker identification and cancer diagnosis due to their biological compatibility, stability, and targeting. Liposomes' multisite and membrane modification abilities should enable sensitive and precise identification of low-abundance, small-sized marker molecules. Liposomes are mostly used for optical (fluorescent) signal amplification, with magnetic particle sensors and electrochemical approaches also effectively work. Modified liposomes can bind cancer-causing molecular antigens for target-specific detection. Encapsulating synthetic probe molecules prevents degradation in vivo, increases probe circulation and targeting, and decreases non-specific molecule capture. Liposome detection technologies have clinical potential and application value due to their excellent recognition efficiency, sensitivity, and specificity. In this section, we summarize the current status, challenges, and development of different liposomal strategies.

Currently, various liposomal medicines, such as Doxil and Onivyde, have been licensed for cancer treatment. Multi-directional research on liposome-based cancer therapies has also progressed to clinical validation [137–140], thereby confirming the advanced and practical efficacy of liposome technology. However, investigations on liposomes for CTBs-targeted detection, including those previously referenced, have been limited to small-scale or prospective surrogate trials. Although they have confirmed their capacity to swiftly and precisely identify and isolate CTBs in blood samples, the practical application remains challenging. Consequently, liposomal probes have to overcome the existing constraints to enhance their application in clinical cancer diagnosis. Firstly, the chemical modification and functionalization techniques for liposome design and manufacturing need optimization for enabling large-scale production [141, 142]. Secondly, the stability and biocompatibility of liposomes as detection probes or carriers in actual applications remain inadequate [143]. Because the susceptibility of lipid molecules to external environments and biomolecules, leading to structural alterations or rapid degradation. Thirdly, existing detection systems that depend on exogenous signal amplification exhibit a deficiency in endogenous selectivity, hence increasing susceptibility to off-target interactions and false positives. Ultimately, the biological toxicity, immunogenicity, and metabolic pathway of the probe must be evaluated [144]. Despite probes being engineered for minimal toxicity, enrichment effects and immunoreaction are unavoidable. The high metabolic clearance and limited imaging penetration will also hinder dynamic in vivo tracking.

In future, to address these issues, innovate high-quality, cost-effective, and non-toxic materials with excellent traits can be used in liposomes synthesized. Furthermore, the progressing environmentally sensitive liposomes can imitate the human body's milieu and respond to disease-related changes by sensing pH, enzyme activity, and temperature variations. Moreover, integrating liposome technology with other molecular recognition techniques may improve cancer detection and real-time analysis. Overall, the key point for liposomes advancement is that a liposome-based "one-stop" testing platform requires development. Malignant tumors require integrated diagnosis and treatment, not only single-level molecular testing. Liposomes' unique programmable structure, multifunctional integration capability, and delivery function allow us to develop all-in-one diagnostic technologies that combine detection and treatment, providing crucial tools for personalized and precise medicine. Therefore, future research should optimize and integrate functional modules, advance clinical translational technologies, and develop disease-specific liposome vectors or probes to promote clinical therapeutic use of liposomes.

Looking ahead, liposome technology will have superior stability, more comprehensive functionality, and be able to function within complex organismal environments with heterogeneity. An integrated diagnostic platform can comprehensively improve liposome utility in oncology. We expect liposome technology to play a more important role within clinical diagnosis and therapy as technology and detection systems improve, allowing for more precise cancer detection and management.

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Authors' contributions
WT: Consulted the literatures, wrote the paper, prepared Tables 1, 2, 3, Graphical Abstract, and Figs. 1, 2, 3, 4, 5, 6, 7; YZ: supervised, provided writing outline; ST: supervised, provided research guidance and critical reviews. All authors contributed to the article and approved the submitted version.

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Declarations

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Competing interests
The authors declare no competing interests.

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