



Computational studies for the development of extracellular vesicle-based biosensors

Maryam Atabay^{a,b}, Fatih Inci^{a,c}, Yeşeren Saylan^{b,*}

^a UNAM-National Nanotechnology Research Center, Bilkent University, Ankara, Turkey

^b Department of Chemistry, Hacettepe University, Ankara, Turkey

^c Institute of Materials Science and Nanotechnology, Bilkent University, Ankara, Turkey

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ABSTRACT

Cancer affects millions of people, and early detection and efficient treatment are two strong levers to hurdle this disease. Recent studies on exosomes, a subset of extracellular vesicles, have deliberately shown the potential to function as a biomarker or treatment tool, thereby attracting the attention of researchers who work on developing biosensors. Due to the ability of computational methods to predict the behavior of biomolecules, the combination of experimental and computational methods would enhance the analytical performance of the biosensor, including sensitivity, accuracy, and specificity, even detecting such vesicles from bodily fluids. In this regard, the role of computational methods such as molecular docking, molecular dynamics simulation, and density functional theory is overviewed in the development of biosensors. This review highlights the investigations and studies that have been reported using these methods to design exosome-based biosensors. This review concludes with the role of the quantum mechanics/molecular mechanics method in the investigation of chemical processes of biomolecular systems and the deficiencies in using this approach to develop exosome-based biosensors. In addition, the artificial intelligence theory is explained briefly to show its importance in the study of these biosensors.

1. Introduction

Cancer is a main cause of mortality all over the world, accounting for approximately 10 million deaths in 2020 (“Cancer Research WHO, 2020,”; Yilmaz and Inci, 2024). Two challenges to combating this disease that scientists face are early detection and effective treatments (Cheng et al., 2019; Erdem et al., 2021; Ginsburg et al., 2017; Guo et al., 2022; Zhou et al., 2017). Traditional diagnostic methods are not efficient for two reasons: (i) they are not practical for repeated screenings, and (ii) they are not accessible to large populations, as well as rural areas have limited access to sophisticated instruments and infrastructure. With this evidence, liquid biopsy holds great potential, and detecting cancer cells from bodily fluids is one of the most promising strategies in the way of cancer diagnostics. Considering the cell level-biomarkers, circulating tumor cells (CTC) and circulating tumor emboli (CTM) however exist very rare—one in a million or billion blood cells (Sagdic and Inci, 2022), requiring more sophisticated devices and tools to detect and isolate for further downstream analyses.

A cancer biomarker is a ‘molecular signature’ and a very important

structure due to the accurate information that carries out the mechanisms underlying the beginning of cancer (Jain, 2017; Park et al., 2017; Wu and Qu, 2015). Cancer-derived extracellular vesicles, and exosomes have been reported as a serious reason in cancer progression and tumorigenesis including cell migration, angiogenesis, immunosuppression, and carry information (Chia et al., 2017; Sharma et al., 2017). Due to these properties, exosomes as the subset of extracellular vesicles are introduced as ideal candidates for developing trusted next-generation biomarkers (Liu et al., 2021).

Biosensors are analytical devices for the detection of analytes by exploiting biological recognition events (Bhalla et al., 2016). They are very sensitive and selective toward structures such as enzymes, peptides, antibodies, and whole cells (Morales and Halpern, 2018). However, the design and optimization of biosensors is challenging for researchers because of the complexity that is in the biosensors-analytes interaction and the structure of biomolecules (Liu and Yue, 2017). Due to the ability that computational methods give the researchers to predict behavior of biomolecules, these tools become powerful in designing and optimizing biosensors (Low et al., 2014). The combination of computational

* Corresponding author.

E-mail address: yeseren@hacettepe.edu.tr (Y. Saylan).

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methods for developing biosensors in experiments enhances the sensitivity, accuracy, and specificity of biosensors (Futane et al., 2022). Computational methods such as molecular docking, molecular dynamics (MD) simulation, density functional theory (DFT), and quantum mechanics molecular mechanics (QMMM) can be used for the prediction of the behavior of biomolecules (Aghajani et al., 2022).

Herein, we review the significance and role of computational methods in developing extracellular-based and exosome-based biosensors. Since exosome as a term has been used in the literature largely, we herein stated such sensors as exosome-based platforms. This knowledge may be exploited to learn about the researches that have been done in computational studies of exosome-based biosensors. Furthermore, because of the deficiencies that are in QMMM studies of this kind of biosensors, this study can be a motivation for future work. We start the discussion with a brief explanation of the contexts of exosome and biosensor. Then, the theory of molecular docking, MD and DFT methods will be explained. And at the end, the theory of artificial intelligence (AI) and its role in the study of exosome-based biosensors will be explained briefly.

2. Extracellular vesicles

Extracellular vesicles (EVs) are created particles from enclosing of cell's lipid bilayer that are released by all cellular organisms into the surrounding environment (Buzas, 2023; György et al., 2011; Parlattan et al., 2023). According to the last nomenclatures and classifications, there are two different particles: (1) exosomes originating from endosomal pathways and (2) ectosomes derived from the plasma membrane (Inci, 2022; Théry et al., 2018; van Niel et al., 2022). According to their sizes, small EVs (diameter of 50–150 nm) hold the majority of this realm whereas EVs (diameter of 200–800 nm) have the smaller numbers. The least abundant population of EVs is large vesicles with a diameter of ≥ 1 μm , even they can carry organelles (Valcz et al., 2019). In general, EVs participate in many biological processes (Liu and Wang, 2023), including differentiation (Meningher et al., 2020; Oh et al., 2015; Stronati et al., 2019), apoptosis (Battistelli and Falcieri, 2020; Paone

et al., 2019), cell motility (Alcántara-Neto et al., 2020; Ferraz et al., 2019; Silva et al., 2016), immunity (Jiang et al., 2022; Kao and Papoutsakis, 2019), proliferation (Ozawa et al., 2018; Takasugi et al., 2017), and reprogramming (Fig. 1) (Brena et al., 2022; Parayath et al., 2020; Zhang et al., 2021).

Since EVs participate closely with several biological processes, they hold crucial clinical potential in diseases (Zhou et al., 2020). In addition to their roles as disease-biomarkers (Couch et al., 2021; van Niel et al., 2022; Van Niel et al., 2018), the researchers utilize EVs for delivering drugs and nucleic acids to the targeted organs or tissues since they carry information from donor-to-recipient cells, (Zeng et al., 2023; Armstrong and Stevens, 2018; Herrmann et al., 2021; Kalluri and LeBleu, 2020; Vader et al., 2016; Walker et al., 2019). Moreover, EVs are being investigated for cancer, inflammatory diseases, and autoimmune disorders therapeutics (Elsharkasy et al., 2020). In this manner, EVs are made by both immune and non-immune cells and then have a significant role in the regulation of immunity. Their therapeutic potential in immune system modulation has been recognized since the first study in 1996 (Robbins and Morelli, 2014), wherein small EVs released by B cells, carrying major histocompatibility complex (MHC) class II molecules, result in immune responses in T cells (Raposo et al., 1996). Notably, dendritic cell (DC) small EVs were reported to induce tumor suppression *in vivo* (Marar et al., 2021). Physical contact and soluble elements are two factors that cancer cells use to inhibit immune cell functionality. In this regard, immune evasion and immunogenic properties are two drivers for these interactions that cancer EVs represent (immune evasion property of cancer-derived EVs) (Hanahan and Weinberg, 2011; Hiratsuka et al., 2006; Kano, 2015; Oskarsson et al., 2011). In addition to cancer EVs can interfere tumor progression; they also show a significant role in accompanying the body in response to cancer (Huang et al., 2017; Kitai et al., 2017; Lin et al., 2020; Rughetti et al., 2014). EVs serve as essential mediators of communication between cells, facilitating the transfer of information (Colombo et al., 2013; Katsuda et al., 2014). Their presence in various bodily fluids, such as cerebrospinal fluid, urine, saliva, and blood (El Andaloussi et al., 2013; Margolis and Sadovsky, 2019), encapsulating nucleic acids, lipids, and proteins,

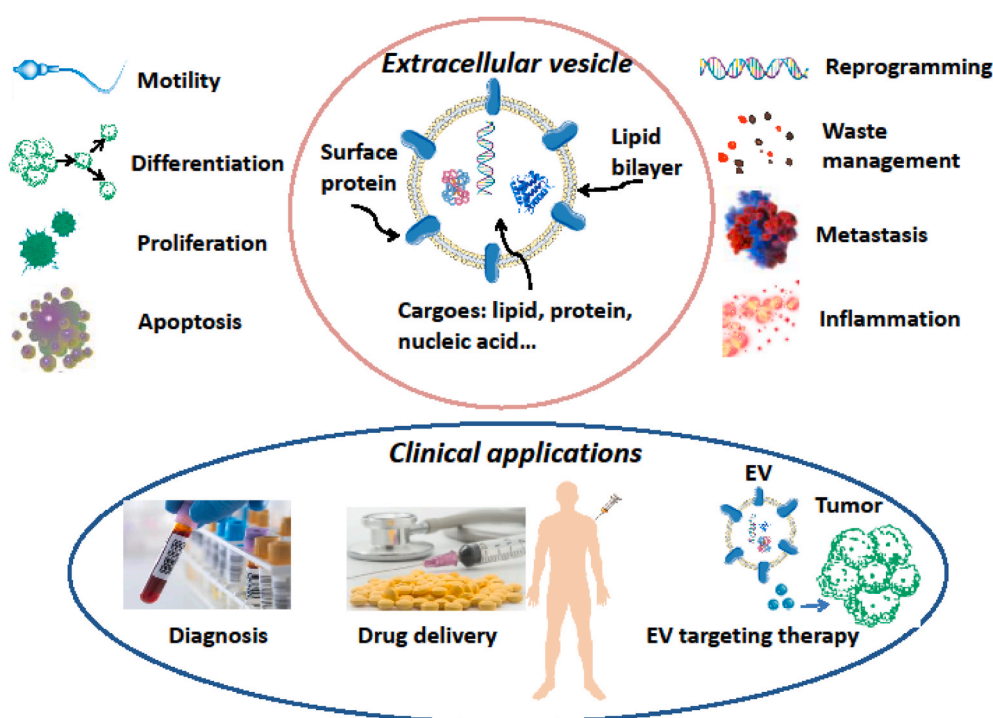


Fig. 1. The general introduction of EVs: the composition, their physiological and pathological processes and clinical applications. Images utilized from Vecteezy and Bioicons (DBCLS) (Liu and Wang, 2023). Copyright 2023, SPRINGER NATURE.

positions EVs as promising biomarkers for early disease recognition and detection. This wide overview underscores the diverse influences of EVs in immune responses, disease progression, and their potential utility as diagnostic biomarkers.

Exosomes, the smallest type of EVs with sizes ranging from 40 to 160 nm, are released through endocytic pathways (Hurley and Green, 2010). Their presence in the biological fluids of cancer patients has positioned exosomes as crucial biomarkers, garnering significant attention from scientists in recent decades. Exosomes play an essential role in reflecting heterogeneous biological changes associated with various conditions and states, including cancer development, progression, response to therapy, and angiogenesis, making them valuable for early cancer diagnosis (Jain, 2017; Liang et al., 2017; Shirejini and Inci, 2022; Wang et al., 2021; Wu and Qu, 2015). As reported in some studies, compared to healthy individuals, cancer patients exhibit higher concentrations of exosomes (He et al., 2018; Tian et al., 2018; Zheng et al., 2023; Zhu et al., 2021). These structures actively influence the metabolism and progression of cancer cells, facilitating communication and the transfer of information between the tumor and its microenvironment. The exosomal biomarkers can be divided into these categories (Soung et al., 2017).

I. **Exosomal surface proteins:** These proteins reflect their origin and variation of the parental cells and are including of: tetraspanins (CD9, CD63, CD81, and CD82) and endosome-associated proteins (annexins, Rab proteins, flotillin, and epithelial cell adhesion molecules (EpCAM)) (Mathivanan et al., 2010).

II. **Exosomal nucleic acids:** These nucleic acids are included in exosomes: microRNAs (miRNAs), tRNAs, mRNAs, lncRNAs, and DNAs. Exosomal miRNAs has been known as widespread and significant biomarkers because of their stability against RNase-dependent degradation. Fig. 2 indicates the biogenesis and characteristics of this type of exosomes that are made during the endosomal network (Gusachenko et al., 2013; Xu et al., 2020).

Based on these features exosomes can be introduced as ideal and credible next-generation biomarkers for noninvasive early cancer detection and assessment of treatment effect (Yu et al., 2022).

3. Biosensors

Biosensors are commonly conceptualized as a three-element system comprising a bioreceptor, a transducer, and a signal-processing unit (Erdem et al., 2023). The measurable signal generated in biosensors originates from the interactions between the analyte and the bioreceptor (i.e. recognition element) (Conroy et al., 2009). They are classified based on two main criteria: (i) recognition elements include nucleic acids, antibodies, enzymes, whole cells, and so on; and (ii) signal transduction methods encompass piezoelectric, electrochemical, optical, and various other approaches (Choi and Yoon, 2023).

Although in recent decades scientists are more desired to do research in the field of exosomes, a need for efficient quantitative methods to recognize exosomes is still seen. Due to the sensitivity of biosensors in the detection of exosomes and their ability in point-of-care (POC)

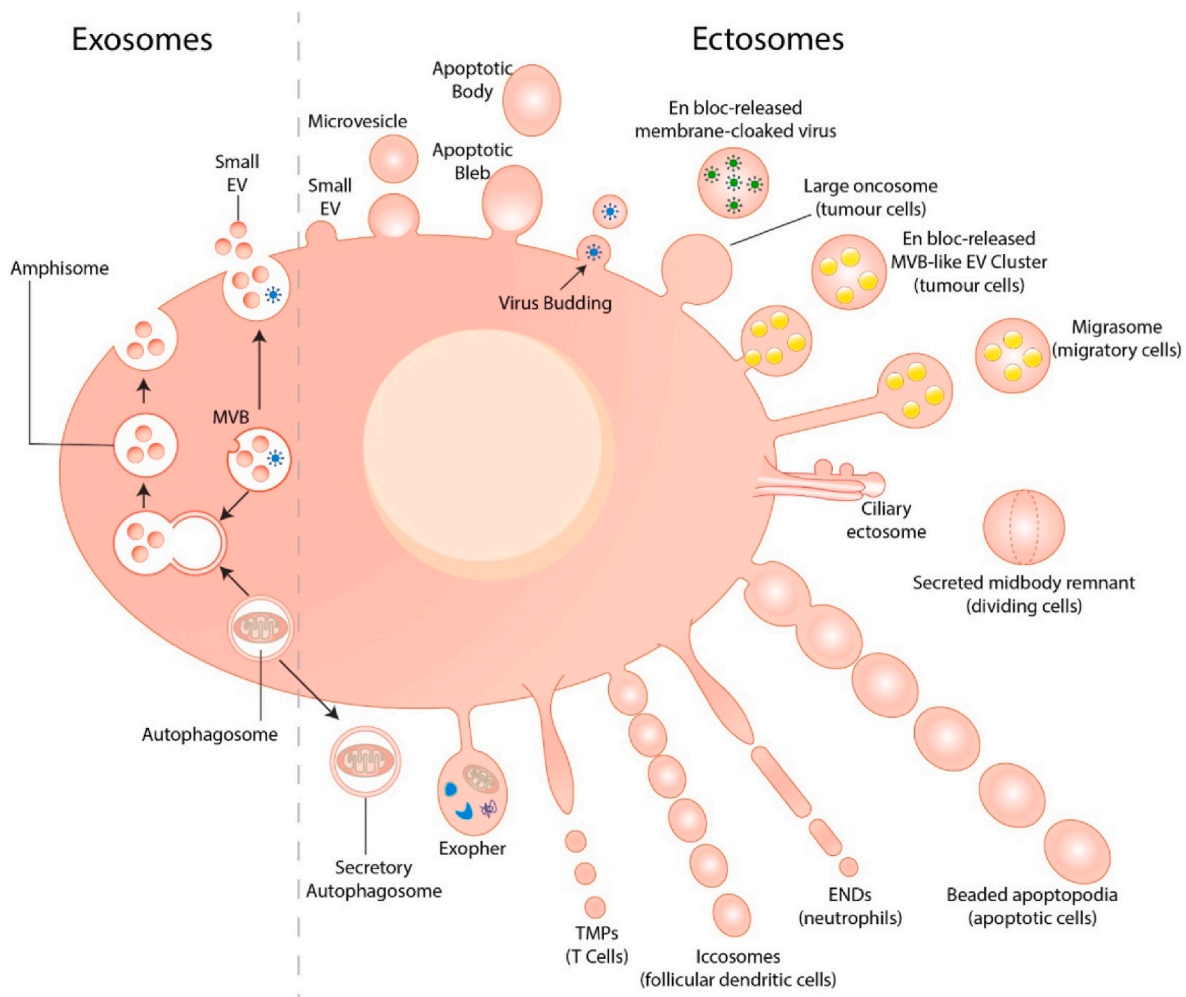


Fig. 2. The heterogeneity of extracellular vesicles (Adopted by Buzas, 2023).

evaluations, they are more desirable in recent years (Aslan et al., 2023; Cheng et al., 2018; Quesada-González and Merkoçi, 2018; Sun et al., 2014; Wen et al., 2017, 2018).

The binding affinity of the recognition element to the target is a factor in choosing the biological recognition elements. Exosomal proteins are effective anti-gens and show the immunoreactions of antigens and antibodies in the development of biosensors (He et al., 2017; Liu et al., 2018). Therefore, exploring detection modalities with the abilities of low limit of detection (LOD), high-throughput screening, small sample volume, and real-time analysis has an important effect on the development of biosensors.

3.1. Optical biosensors

This section is mainly positioned to present and discuss the studies focusing on Raman scattering, fluorescence, colorimetry, and surface plasmon resonance (SPR) for the recognition of miRNAs or exosomal proteins (Xu et al., 2020).

Surface-enhanced Raman scattering biosensors: Remarkable single molecule-level sensitivity and insensitivity to quenching are two properties of surface-enhanced Raman scattering (SERS)-based biosensors that made them applicable for the detection of exosome and have two system models: (i) solution-based and (ii) solid-based. They have some distinguished properties such as single molecule-level sensitivity and insensitivity extinguishing (Cheng et al., 2019; Derin and Inci, 2022). In comparison to fluorescence or SPR, this biosensor can detect specified spectral signals in a changeable and complicated biological environment (Zong et al., 2013). As an example of the application of this method, Wang et al. study can be mentioned that they proposed a SERS based method to detect HER-2, CEA, and PSMA proteins of colorectal, prostate, and breast cancer cells. For the SKBR3, T84, and LNCaP exosomes, respectively they got the LOD values of 32, 73, and 203 exosomes/ μL . To investigate the practical application of this method they used the samples of patients' real blood for the mentioned cancer diseases (Weng et al., 2018). Furthermore, Tian et al. presented a convenient and highly sensitive SERS based method combined with immunoaffinity, portable Raman devices, and SERS nanoprobe for accurate quantification and isolation of CD9 protein of human liver carcinoma cells. They evaluated this biosensor based on SERS in optimum conditions and achieved the LOD of 27 particles/ μL . They investigated the lifetime, stability, and reproducibility of their propped biosensor and indicated that the SERS signal of the sandwich-type immunocomplex has excellent reproducibility while, after 24 h its sensitivity could keep 95.3% intensity of the initial SERS and it decreased notably from the third day. To show the feasible application of their biosensor, they studied three liver cancer patients' serum samples and three healthy serum samples (Tian et al., 2018).

Fluorescent biosensors: Fluorescent biosensors have been developed for the recognition of exosomes and because of their high sensitivity, show great advantages. Their fluorescent signal usually depends on the applying of fluorescent proteins, fluorescent dyes, fluorescent nanoparticles, or fluorophores. They are divided into three system models: solution-based, paper-based, and microplatform-based (Cheng et al., 2019). Chen et al. using the luminescence resonance energy transfer (LRET) method studied the recognition of CD63 protein extracted from the source of human liver cancer cells (Hep G2) in the body of liver cancer patients. In this work, they applied a simple paper relying aptasensor based on LRET. They monitored the variations using the home-made image system and the value of achieved LOD was 1.0×10^4 – 1.0×10^8 particles/ μL (Chen et al., 2018a). Liu et al. expanded an immune-sorbent evaluation for qualification of exosomes using droplet microfluidics and validated their approach by applying in breast cancer patients' plasma samples to quantitative detect of exosomes. Sandwich ELISA complexes that are characterized by an enzymatic reporter with the ability to produce a fluorescent signal are used and they immobilized exosomes on magnetic microbeads. They could achieve the LOD of

$\sim 10^{-17}$ exosome complexes/ μL . To recognize exosomes they performed droplet digital ExoELISA in serum samples of 5 patients with benign breast disease, 5 healthy samples, 12 patients with breast cancer, 5 patients with benign breast disease, and 2 patients with breast cancer after surgery (Cohen et al., 2008).

Colorimetric biosensors: Colorimetric biosensors show great importance for POC applications due to their properties such as simple operation and convenient readout. They have two models: paper-based and solution-based. According to the concentration of colored compounds in the solution, this evaluation can label sample matrices via the color rendering principle and it can be seen with the naked eye without extra analysis equipment (Cheng et al., 2019). Chen et al. presented a ZnO nanowires coated a 3D scaffold device covered by the ZnO nanowire array for the detection of the CD63 protein in breast cancer cells. The 3D derives the fluid flow and ZnO nanowire array provides a large surface area and immobilizes exosome-specific antibodies as the capture of exosomes enhances at a high flow rate. UV-vis spectrometry or microplate reader was applied for quantitative measurement of exosomes and a value of 2.2×10^4 particles/ μL was achieved for the least detectable concentration. For the detection of exosomes from clinical samples, they pumped 100 μL serum (plasma) into the ZnO-chip device that functionalized with an antibody (anti-CD63) and then detected using colorimetric ELISA (Chen et al., 2018). Liu et al. using AuNP-based colorimetric assay generated a unique surrogate DNA signal for the detection of LMP1, EGFR proteins from nasopharyngeal carcinoma (NPC) cells and plasma of patients with NPC. Their method has properties such as high sensitivity, convenience to use, a simple strategy for detection, and performance in a short time. Their reported value of LOD was 10^2 particles/ μL , LMP1 with an accuracy of 0.956, and EGFR with an accuracy of 0.906 (W. Liu et al., 2018).

Surface plasmon resonance (SPR) biosensors: These biosensors can sense biological binding events and interactions happening until 200 nm depth of a gold layer (Cheng et al., 2019). It is a label-free method that does not need boring steps of sample handling (Xu et al., 2020; Erbaş and Inci, 2022; Erdem et al., 2019). To increase the sensitivity of plasmonic biosensors, in 2018, Zhu et al. designed novel 3D plasmonic photonic crystal (PPC) nanostructures with point-defect cavities to detect EpCAM protein of fibroblast L cells. Their 3D PPC nanostructures for the resonance peak at 1232 nm showed the highest sensitivity of 1376 nm RIU^{-1} and the highest figure of merit (FOM) of 11.6 for the resonance peak at 564 nm. They could recognize 10 μL of fibroblast L cell-derived exosomes with a concentration range of 1×10^4 – 1×10^{11} particles/mL (Zhu et al., 2018). Liu et al. developed a compact SPR biosensor with modulated intensity and conventional SPR sensing mechanism for the detection of EGFR, and PD-L1 proteins of lung cancer. In this work to study the clinical efficiency of SPR biosensors, five patients' serum samples and five healthy samples were prepared. The sensitivity and resolution values are $9.258 \times 10^3\%/\text{RIU}$ and $8.311 \times 10^{-6} \text{ RIU}$, respectively (Liu et al., 2018b).

3.2. Electrochemical biosensors

Electrochemical biosensors produce electrical signals during the detection of a biomolecule (impedance, current, and potential) (Cheng et al., 2019). The advantages like higher sensitivity, cost and time-effective procedure, and needing lower sample quantity led to more attention to these types of biosensors (Seifati et al., 2018; Shoaie et al., 2019). Therefore, due to these properties besides the benefits of the too small size of exosomes, electrochemical biosensors play an important role in the detection of exosomes in medical samples (Hochendoner et al., 2018; Im et al., 2018; Wang et al., 2018). Amperometric, impedimetric, and voltammetric are electrochemical detection techniques for exosome detection (Xu et al., 2020).

Amperometric biosensors: The amperometric biosensors are based on an enzyme's redox activity and measure the current of electrodes. Chronoamperometry is a formative of amperometric methods. As the

generated signal response is near the surface of the sensor electrode, the properties of the electrode affect these biosensors (Bollella and Gorton, 2018; Monteiro and Almeida, 2019; Topkaya et al., 2016). Because of the catalytic activity of enzymes, they are accelerator in chemical reactions and this property of these macromolecules improves the sensitivity of biosensing systems (Shoaie et al., 2019). In 2016, Jeong et al. proposed a unified magneto-electrochemical amperometric biosensor for screening of exosomes. The sensor works according to the combination of magnetic selection and electrochemical detection. Exosomes get captured and labeled by magnetic beads and then are detected via electrochemical sensing. To demonstrate clinical feasibility they isolated EVs directly from plasma (serum) of ovarian cancer patients and their method's sensitivity was $<10^5$ vesicles (Jeong et al., 2016).

Impedimetric biosensors: In impedimetric methods, the impedance parameter of a system is measured. In the electrochemical biosensing field, the most important impedimetric biosensor is electrochemical impedance spectroscopy (EIS). An excessive advantage of this method is being a label-free detection method. This technique is sensitive for the analysis of the biorecognition events and types of immunosensors and aptasensors of these biosensors are more studied (Bahadir and Sezgin-türk, 2016; Bertok et al., 2019). In 2018, Kilic et al. manufactured an electrochemical sensor to label-free measure of EVs levels in breast cancer. For the selectivity test, an abundant protein in the blood (RhD) was used. The detection was carried out using Differential Pulse Voltammetry (DPV) (with an LOD value of 379 EV/mL) and Electrochemical Impedance Spectroscopy (EIS) (with an LOD value of 77 EV/mL) (Kilic et al., 2018). Li et al. introduced the utilization of ultrasensitive EIS to quantify tetraspanin and syntenin exosome markers of serum. They used the ultrasensitive electrochemical impedance spectroscopy and quantified the exosome-specific biomarker. The LOD of (1.9×10^5 particles/mL) for intact exosomes and (3–5 pM) for internal exosomal syntenin levels were the results of their method (Li et al., 2017).

Voltammetric biosensors: In this method current as a performance of electrode potential is measured. Voltammetric biosensors have some technical advantages such as in comparison to other techniques there is no limitation for the calculation of ions and molecules (Scholz, 2015). Boriachek et al. reported an electrochemical biosensor for the recognition of breast and colon tumor-specific exosomes. Using quantum dots as signal amplifiers they reported a stripping voltammetric immunoassay for detection of exosomes. As breast and colon cancer markers they used antibodies of (CdSe quantum dot (CdSeQD) functionalized-biotinylated HER-2 and FAM134B). The sensitivity of their method was (100 exosomes/ μ L) in serum samples of nine patients with colorectal adenocarcinoma (Boriachek et al., 2017). Yadav et al. applied a sandwich strategy assay to develop electrochemical biosensors. Using the DPV method they investigated quantifying breast cancer cell-derived exosomes. Their evaluation has a two-step design where a generic antibody (i.e. tetraspanin biomarker, CD9) initially captures exosome populations, and then subsequent detection of capture exosomes is done by using a cancer-specific antibody (growth factor receptor 2 (HER-2)). They achieved an LOD of 4.7×10^5 exosomes/ μ L and a relative standard deviation of $<4.9\%$ (Yadav et al., 2017).

Moreover, exosomes can be detected when electrically polarized due to their ability to store electrical energy. It should be mentioned that the electrical methods are applied to few researches so far and include: electric field-induced release and measurement (EFIRM), field effect transistor (FET), and oxygen-plasma-treated reduced graphene oxide (rGO) (Xu et al., 2020).

Electric field-induced release and measurement-based biosensors: In the electric field-induced release and measurement (EFIRM) method the exosomal RNA/proteins biomarkers can be monitored by releasing the contents of the exosome structure. In this method according to CD63 affinity-based magnetic, exosomes are extracted, and then releasing, collecting, and incubating of the exosome-harbored biomolecules are done by applying the square wave electrical field (csw E-field) (Wei

et al., 2009a; 2009b). Wei et al. proposed a way for the detection of harbored exosomal biomolecules using the EFIRM technique. In their method, exosomal proteins and RNA are rapidly released when they have been subjected to a non-constant electrical field. In addition to detection in blood, they could demonstrate tumor exosomes detected in saliva (Wei et al., 2013).

Field effect transistor (FET)-based biosensors: This kind of electronic biosensor is pivotal because of properties such as high sensitivity, the ability of simple signal readout, short response times, and label-free recognition. Nevertheless, in high ionic intensity solutions, the clinical applications of these biosensors were restricted (Arya et al., 2015; Chen et al., 2011). Pulikkathodi et al. reported the ability of a high-field modulated AlGaIn/GaN high electron mobility biosensor for the detection of CD63 protein. Their biosensor can identify EVs in solution with high ionic strength and doesn't need any additional reagents, provides high sensitivity with a range of detection of EVs (10^7 – 10^{10} EV/mL) (Pulikkathodi et al., 2018).

Oxygen-plasma-treated reduced graphene oxide-based biosensors: The remarkable electrical property and easy modification and functionalization of the surface of graphene led to its widespread usage in developing electrical biosensors. In this regard due to the numerous advantages of reduced graphene oxide (rGO) such as simple synthesis, good biocompatibility, and large accessible surface area, this material is permitted for graphene-based biosensors (Balasubramanian and Kern, 2014; Ohno et al., 2010; Schedin et al., 2007). In 2017, Chae et al. detected Exosomal A β peptides of Alzheimer's disease using oxygen plasma treatment. To have better surface reactivity of rGO and to enhance its sensing performance in biomolecular detection, they performed oxygen plasma treatment. Their results showed that their method improves the sensor performance. Their proposed biosensor showed 3.33-fold greater responses in target-specific recognition (Chae et al., 2017).

4. Computational methods

4.1. Molecular docking

Molecular docking is the most common method that has been used in structure-based drug design since the early 1980s for predicting the position and affinity of a small molecule as a ligand on the structure of a macromolecule as a receptor (Astalakshmi et al., 2022; Roche et al., 2015; Tao et al., 2020). The first step in docking calculation is finding the 3D structure of biological molecules. They can be the structure of protein, DNA or RNA. The structure of these macromolecules can be easily prepared from the Protein Data Bank (PDB) (Saur et al., 2021), but if there is no defined 3D structure of the target, computational prediction methods, such as ab initio modeling can be used to obtain the 3D structure (Torres et al., 2019). The required ligand structure can be obtained from small molecule databases such as ZINC and PubChem (Irwin and Shoichet, 2005; Kim et al., 2016). If their 3D structure is not directly available, software such as ChemDraw and Avogadro help obtain their structure. A successful docking methodology needs to find the ligand situation properly and calculate physical- and chemical-interactions (Kolb and Irwin, 2009). Fig. 3 presents an example of the molecular docking process. According to the flexibility of the structure of receptor, there are different types of molecular docking (Ferreira et al., 2015):

Rigid docking: In rigid docking, the structure of ligands and receptors does not change, and it is appropriate for large systems such as protein-nucleic acid and protein-protein interactions (Tao et al., 2020; Yan and Wang, 2013). This method is the simplest and does not need multiple calculations.

Semi-flexible docking: In semi-flexible docking, the conformation of the ligand changes while the conformation of the receptor remains unchanged. This method is appropriate for the interactions of small molecules (ligands) with the structure of macromolecules such as proteins

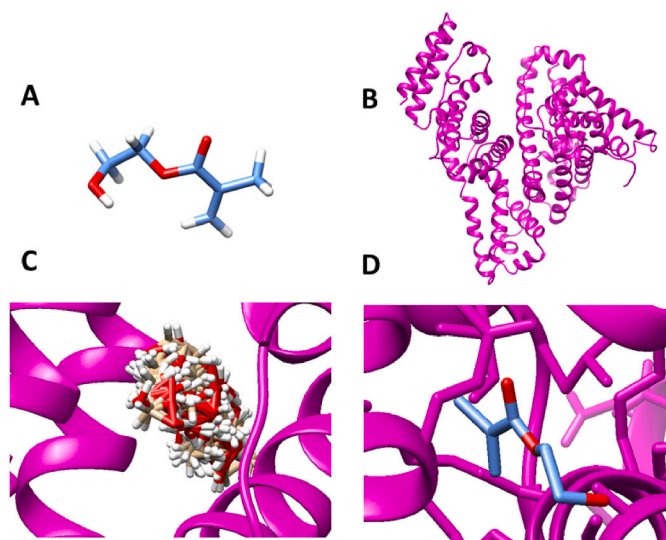


Fig. 3. The molecular docking processes. A) the ligand's structure, B) the receptor's structure, C) the docked ligand in the receptor's cavity, and D) the most likely binding conformation and related intermolecular interactions.

and nucleic acids (Liu et al., 2016; Morris et al., 1998; Tiwari and Mohanty, 2013).

Flexible docking: During this process, both the structure of the ligand and receptor can be easily modified. Flexible docking is used to study the interaction between molecules accurately and due to the increase of ligand and receptor variables with increasing the number of atoms, this method is too complicated (Jiang et al., 2016).

Molecular docking aims to computationally simulate and complete an optimized conformation and, in this way, the free energy of the overall system is minimized (Roy et al., 2018). Finding the best position and binding conformations needs steps including (1) accurate prediction of the interaction energy, and (2) finding the large conformational space (Shahbaaz et al., 2018). Furthermore, most docking methods assume that charge distribution and particular protonation states don't change between their bound and unbound states (Torres et al., 2019). Molecular docking programs perform the search algorithm and evaluate the conformation of ligands until the convergence to the minimum energy is achieved (Kapetanovic, 2008). Systematic algorithms and stochastic methods are applied in molecular docking to explore all degrees of freedom of ligand during the search (Pagadala et al., 2017; Huang and Zou, 2010).

4.2. Molecular dynamics simulation

Molecular dynamics simulation predicts how a molecular system such as a protein in water system will move over time according to conventional physical chemistry/thermodynamic models (Hollingsworth and Dror, 2018). It is a practical method for understanding of physicochemical properties of structures at the molecular scale and offers molecular dynamic information including molecular interactions (Zendehboudi, 2023). Newton's law of motion (Hollingsworth and Dror, 2018) is solved for every atom in the system; the position and velocity of molecules are derived during the simulation and thermodynamic and kinetic properties of the system can be determined. In MD simulation for every simple atomic system Newton's equation of motion is defined:

$$m_i \ddot{r}_i = f_i \quad f_i = -(\partial / \partial r_i)U \quad (1)$$

where m_i : atomic mass, r_i : atomic coordination, r_i : forces acting on every atom, and U : potential function (Gubbins, n.d.; Maitland et al., 1983; Sprik, 1993; Stone, 2013). MD simulation relies on empirical

approximations called force fields to evaluate the interaction and potential energy between particles (Kukol, 2008). For some structures such as DNA, RNA, peptides, and protein, force field parameters are optimized, but for other structures in which these parameters are not available, these molecules need to be parameterized (Padhi et al., 2022). The common force fields for large systems are CHARMM (MacKerell et al., 2000; Bayly et al., 1995), AMBER (Bayly et al., 1995), and GROMOS (Oostenbrink et al., 2004). In addition, the force field of (UFF) (Rappé et al., 1992), MMFF94, or MFF94x (Halgren, 1996) are universal for the smaller molecular systems (Shakib, 2021). Equation (2) defines the CHARMM force field (Atabay et al., 2022):

$$U_{ij} = 4\epsilon_{ij} \left(\left(\sigma_{ij}/r_{ij} \right)^{12} - \left(\sigma_{ij}/r_{ij} \right)^6 \right) + q_i q_j / 4\pi\epsilon_0 r_{ij} \quad (2)$$

Where:

$$\sigma_{ij} = (\sigma_i + \sigma_j) / 2 \quad \epsilon_{ij} = (\epsilon_i \cdot \epsilon_j)^{(1/2)} \quad (3)$$

where, ϵ_{ij} , σ_{ij} and r_{ij} are the depth of LJ potential, the LJ core parameter, and distance between particles i and j ; ϵ_0 is the susceptibility of vacuum and q is the partial atomic charge

Regarding force fields, the summation of bonded and nonbonded interactions gives the total potential energy function of the system. The bonded potential is related to the directly bonded atoms and includes bond stretching, angle bending, and dihedral angle torsion. In contrast, the non-bonded potential is defined between atoms that are not directly bonded (Seyyedattar et al., 2019). MD simulation offers valuable information, such as temperature, pressure, flexibility, kinetic and potential energy, viscosity, diffusivity, and radius distribution function. Some of these parameters can be obtained directly through the particle trajectories (e.g., potential energy) and some other thermodynamic properties can be calculated from the system's parameters, e.g., kinetic energy that can be obtained from the system's temperature (Seyyedattar et al., 2019).

Similar to other molecular methodologies, MD simulation has some weaknesses and strengths. MD approach has the ability to investigate the behaviour of molecules in complex systems such as proteins, enzymes, and polymers. It can be applied to analyze a wide range of temperatures and pressure under conditions that are more controllable than laboratory tests' conditions (Ren et al., 2022). This technique would be the best strategy for studying the thermodynamics, structural, and mechanical properties of rare and expensive molecules. Yet beside all these advantages of MD simulation, needing a strong CPU and long simulation time due to the large number of atoms are disadvantages of this method (Seyyedattar et al., 2019). In the following, some limitations of MD simulation that are better to consider when using this technique have been discussed.

- **Force field parameters:** The correct definition of potential energy affects the results' reliability. The accuracy of force field parameters is debated because they are based on experimental data and approximation. To calculate potential energy, the dielectric constant of vacuum is used, while molecules are surrounded by aqueous solutions with different dielectric constants. In addition, intermolecular hydrogen bonds are not considered in most force field parameters.
- **Time and size scales:** The length of time step, duration of simulation, and number of particles can be the reason for errors and problems in MD simulation. Due to the experimental investigations that happen at the microscopic scale then long-time simulations should be applied to obtain reliable results. The time step length should be in the order of several femtoseconds (10–15 s). Furthermore, in this technique, computational cost is faster but less accurate (Zendehboudi, 2023).
- **Use of classical mechanics:** In MD simulation individual atoms are treated as soft balls (ball and stick model), bonds are elastic sticks

and no bond formation or breakage happens (Padhi et al., 2022). Therefore, MD simulation cannot capture the quantum effects including chemical bonds, the existence of noncovalent intermediates, and specific heat drops in crystals.

4.3. Density functional theory

Density functional theory (DFT) is a computational quantum mechanical tool to study the electronic structure of atoms, molecules, and solids (Oselusi et al., 2024). This method is developed to solve the Schrödinger equation. Many properties like electrode/electrolyte materials, band structure, and structural stability can be calculated accurately with the DFT approach (Ceder et al., 2011; Kang et al., 2006; Kushwaha et al., 2022; Ladha, 2019; Tianran et al., 2013). In the DFT method, the electron density as a fundamental quantity describes the interaction of electrons and provides the property of ground state. In 1960, DFT was formulated on the Hohenburg-Kohn theorem, and in this theory the ground state density is defined in terms of single-particle orbitals (Filippi et al., 1996):

$$\left[- (1/2) \nabla^2 + V_{\text{ext}}(\mathbf{r}) + \int (\rho(\mathbf{r}') / |\mathbf{r} - \mathbf{r}'|) d\mathbf{r}' + V_{\text{xc}}([\rho]; \mathbf{r}) \right] \psi_i = \varepsilon_i \psi_i \quad (4)$$

where:

$$\rho(\mathbf{r}) = \sum_{i=1}^N |\psi_i(\mathbf{r})|^2 \quad (5)$$

The electron density $\rho(\mathbf{r})$ is constructed by summation over the N lowest energy orbitals (N is the number of electrons) and V_{ext} is the external potential. The term in brackets is the Kohn–Sham Hamiltonian and appropriate methodology and basis sets are needed to express the solutions of that. The exchange-correlation potential $V_{\text{xc}}([\rho]; \mathbf{r})$ is a functional parameter for exchange-correlation energy $E_{\text{xc}}[\rho]$. Equation (6) defines the total energy of system (E):

$$E = -1/2 \sum_{i=1}^N \int \psi_i \nabla^2 \psi_i d\mathbf{r} + \int \rho(\mathbf{r}) V_{\text{ext}}(\mathbf{r}) d\mathbf{r} + 1/2 \int \int \rho(\mathbf{r}) \rho(\mathbf{r}') / |\mathbf{r} - \mathbf{r}'| d\mathbf{r} d\mathbf{r}' + E_{\text{xc}}[\rho] \quad (6)$$

and

$$E_{\text{xc}}[\rho] = E_x[\rho] + E_c[\rho] \quad (7)$$

where: $E_x[\rho]$ and $E_c[\rho]$ are exchange and correlation contributions separately.

The calculations of ground state using the DFT approach give us the energy (E) and density $\rho(\mathbf{r})$ of the system and consequently, any quantities that can be extracted from them (e.g., the ionization potential) without using any experimental parameters (Jones, 2015). For most applications, energy (E) is considered a function of the nuclear coordinates and most of the properties of molecules or materials such as angle, bond length, all reaction energies, vibrational frequency and transition state barriers, etc. can be derived only from this quantity (Burke, 2012). The electron density in the ground state of atoms falls from the nuclei as a function of distance. The electron density in molecules and solids has small departures from the densities of their constituent atoms. These changes in density distribution led to the energy differences such as binding, ionization, and cohesive energy that are the focus of much DFT work (Jones and Gunnarsson, 1989). One of the most fundamental properties of biomolecules is their structure and in addition, the relation of their structure to biological function. DFT approach is able to rapidly and accurately optimize the structures, e.g., DFT code

is used to calculate the electrostatic potential of the aldose reductase enzyme's surface and the result is in good agreement with high-resolution x-ray diffraction data (Cole and Hine, 2016). Hardness as a key property of hard coatings can be conceived as resistance of materials. It is defined by bond strength. Hardness is governed by the electronic structure which can be predicted using DFT theory. Here, DFT enables atomic scale understanding of the phase stability, interfaces for the elastic properties, formation of defect structures, and enhancement of toughness (Music et al., 2016). Another usage of DFT calculations is that it gives a meaningful description of the electronic structure, magnetic interactions, phase stability, and crystal lattice of magnetically doped oxide systems that are important because of their potential ferromagnetic behavior and can be utilized for various spintronic devices (Assadi and Hanaor, 2013). Beside all of these useful roles of DFT, there are some limitations when using this method.

- DFT is a reliable technique to study atomic features; however, it is expensive in terms of computational resources and it is not feasible for large-scale simulations (Padhi et al., 2022).
- Although the DFT method is the quickest way of quantum calculations of electronic structure, it is too slow for liquids.
- DFT has provided approximations that for some problems they work, yet fail for the other problems (Burke, 2012).
- Band structure calculations based on DFT with exchange-correlation potentials are known to underestimate the band gap of insulating materials and semiconducting (Morales-García et al., 2017).

In the following, some information has been collected briefly in Table 1 about these simulation and computational methods.

5. Computational methods in exosome biosensors and exosome-mimicking systems

5.1. Molecular docking and molecular dynamics simulations for the applications of exosome biosensors and exosome-mimicking systems

The membrane of tumor-derived exosomes has Ptd-LSerIn (Théry et al., 2002) that can bind to immunoglobulin- and mucin-domain containing molecules (Tim-4) (Miyayoshi et al., 2007). Hosseini et al. reported the design of single-walled carbon nanotubes (SWCNT) as nano-biosensor for early detection of cancer. Using MD simulations they studied the interaction and the binding mechanism of SWCNT and Tim-4 to the tumor-derived exosomes phosphatidylserine (Ptd-L-Ser) receptor. This study confirms that Tim-4 keeps its biological ability when it interacts with SWCNT and confirms the affinity between Tim-4 and Ptd-L-Ser receptor (Hosseini et al., 2016a). Li et al. described a polymer-sorted carbon nanotubes (CNTs) biosensor to detect the transmembrane protein of breast cancer exosomes (MUC1). Because it is not easy for this structure to make a stable functionalized structure with biomolecules, they used poly-lysine (PLL) to modify CNT. In order to confirm the binding stability of PLL and CNTs, this group run the MD simulation and investigated the interaction between them. The calculated interaction energy between these two molecules along the simulation was -73.01 kcal/mol which indicates the stable binding of PLL to CNTs (Li et al., 2023). Liu et al. presented a smart polymer: poly (N-isopropylacrylamide-co-N-acryloxysuccinimide (PNN) for studying exosomal mRNAs as cancer biomarkers. In the process of synthesis of polymer, they used the MD simulation to reveal how phase transition is dependent on temperature. Their studies showed that PNN has a

Table 1
Some properties of simulation and computational methods.

Molecular Docking	Definition	Is a method for prediction of the preferred orientation of two molecules (ligand and target) that are bound to each other to form a stable complex (Lengauer and Rarey, 1996).
	Theory and Method	Two approaches are popular within molecular docking: a) describing of protein and ligand as complementary surfaces, b) simulation of the actual docking process and calculation of ligand-protein pairwise interaction energies using search algorithms (Feig et al., 2004; Goldman and Wipke, 2000; Meng et al., 1992; Morris et al., 1998; Torres et al., 2019).
	Application	In the field of drug design, to predict pollutants degraded by enzymes, to predict the position and orientation of ligands binding to proteins, using for quick screening of large databases of potential drugs in silico (Basharat et al., 2017; Suresh et al., 2008).
	Limitation	1. Scoring functions are not trustworthy enough to give accurate binding energies (Sethi et al., 2019; Yuriev et al., 2011), 2. Unsuccessful to make the correct prediction of binding mode (Verkhivker et al., 2000), 3. No identifying of water molecules in the binding pocket during the docking process (Spyrakakis and Cavasotto, 2015), 4. One major challenge in the field of docking is rigid receptor because a protein can adopt many different conformations when it interacts with a ligand (Shoichet et al., 1999).
MD	Definition	Is a method for studying the physical movements of atoms and molecules (Baez and Peter May 2012).
	Theory and Method	For a system of interacting particles, Newton's equation of motion is numerically solved for the determination of the trajectories of atoms and molecules, in addition, forces and potential energy between particles are calculated using interatomic potentials or molecular mechanical force fields (Baez and Peter May 2012).
	Application	Is common in biochemistry and biophysics: refining 3-dimensional structures of proteins and other macromolecules, studying the motions of proteins and nucleic acids, ligand docking, ligand-protein conformations, examining the dynamics of atomic-level phenomena: thin-film growth and ion-supplantation, for pharmacophore development and drug design (Choudhury et al., 2015; Hatmal et al., 2016; Koehl and Levitt, 1999).
	Limitation	1. Limitations are mainly in the length of possible simulation and the size of the system (Berendsen, 1999). 2. The force fields used require further refinement (Choi et al., 2018). 3. Where the effects of quantum are important, these simulations are poorly suited to the systems (Hong et al., 2011). 4. There is no accepted polarizable force field therefore; those force fields that are available are rare (Jorgensen, 2007).
DFT	Definition	Is a computational quantum mechanical modeling method used in condensed-matter physics, computational physics, computational chemistry, and solid-state physics (Assadi and Hanaor, 2013).
	Theory and Method	Solving the Kohn–Sham equations by defining the exchange and correlation interactions to investigate the electronic structure (or nuclear structure) (principally the ground state) of many-body systems (Assadi and Hanaor, 2013; Hanaor et al., 2012).
	Application	Applied for synthesis-related systems and processing parameters, studying the magnetic and electronic behavior in ferroelectrics and dilute magnetic semiconductors, studying the

Table 1 (continued)	
	effects of dopants on phase transformation behavior in oxides, the discovery of new hard coatings (Assadi and Hanaor, 2013; Music et al., 2016).
Limitation	1. DFT calculations underrate the barriers of chemical reactions, the energies of dissociating molecular ions, the band gaps of materials, and charge transfer excitation energies (Cohen et al., 2008). 2. They overrate the response to an electric field in molecules and the binding energies of charge transfer complexes (Cohen et al., 2008).

property of sensitive thermal-responsive phase transition (Liu et al., 2022). Hosseini et al. defined an engineering route to fabricate some surface-functionalized nanoparticles that can imitate the targeting mechanism of tumor-derived exosomes. They investigated the interaction between ligand (TIM4) and receptor (phosphatidylserine (PS)) using the MD simulation. According to their results, looks that the complex of TIM4/PS releases enough free energy to induce endocytosis. The selected ligands were studied to confirm their improved targeting efficacy. Their work was a spotlight to show the importance of simulating methods for next-generation nanomedicine (Hosseini et al., 2016). By employing tau biosensor cells, Bilousova et al. showed that cambinol inhibits the activity of neutral sphingomyelinase (nSMase2) enzyme and can reduce tau spread from donor cells and showed that this inhibitor is much better than the nSMase2 inhibitor (GW4869). They reported that the expansion of tau oligomers taken from synaptosomes of Alzheimer's disease patients was inhibited by cambinol. They studied the position of combinol in nSMase2 using molecular docking and the interactions between combinol and nSMase2 using MD simulation. Their analysis indicates that combinol can select the DK-switch in the active site of nSMase2. nSMase2 is a gatekeeping enzyme that takes part in the production of ceramide-mediated exosomes (Bilousova et al., 2018).

On the other hand, there are some studies focusing on exosome-mimicking systems such as liposomes. For instance, Dominguez-Arca et al. investigated the new structure of cationic liposomes by mixing dipalmitoylphosphatidylcholine (DPPC) lipid with tetradecyltrimethylammonium bromide gemini surfactants. Their new formula of cationic liposome introduces their new application in biosensing, drug delivery, and vaccines. They used the MD simulation to study the interaction between the lipid bilayer of DPPC and gemini surfactants (Dominguez-Arca et al., 2022). Wallner et al. by studying the binding interaction of protein and liposome using the MD simulation showed that all liposome formulations formed stable complexes with immobilized proteins and their results introduced the biosensor platform for drug screening and drug discovery (Wallner et al., 2013). Using the MD simulation Holme et al. investigated how changes in the lipid composition and packing affect the stability of vesicles. Their findings report that liposome-based diagnostic and technologies of drug delivery for cases that phospholipase dysregulation have a role in diseases (Holme et al., 2018). Li et al. using coarse-grained MD simulation studied the mechanical and physical interaction between hydrophobic nanosheets and lipid liposomes. Their results facilitate a molecular-level understanding of the cytotoxicity of 2D nanomaterial in cells which is challenging for their promising application in biosensing and drug delivery (Li et al., 2019). Diverse biometric applications of membrane proteins indicate an important role of supported lipid bilayers (SLBs). Accordingly, in order to learn the mechanism of liposome spreading Kong et al. examined the effects of substrate charges on unilamellar liposome spreading based on coarse-grained MD simulation. Their results reveal the important role of electrostatics interactions on liposome spreading and give knowledge about (SLBs) that hold great promise for biosensing devices (Castellana and Cremer, 2006; Kong et al., 2016). Parchekani et al. using coarse-grained MD simulation developed a liposome model system and showed that their model can be useful in finding out the

mechanism and function of biological cell membranes as potential drug carriers to design and introduce optimal, appropriate, and biocompatible liposomes (Parchekani et al., 2022).

5.2. Density functional theory for the applications of exosome biosensors and exosome-mimicking systems

Chen et al. developed a dual-signal amplification electrochemical biosensor. Their biosensor was used to recognize exosomal microRNA-21 (as good potential in cancer recognition). They used a self-assembled electrode integrated from hexanethiol poly-adenine and cholesterol-modified DNA. They compared the interaction of Au with both adenine and hexanethiol poly-adenine using DFT computations, and their results showed that hexanethiol poly-adenine binds to Au via Au-S bond (Chen et al., 2022). In 2021, Xia et al. for the first time studied the visible-light-induced oxidase mimic activity of 10-methyl-2-amino-acridone (MAA) that can be changed by Cu^{2+} and DNA. They developed a strategy to detect the exosomal microRNA-21 (miR-21). DFT computations were used to better understand the electronic features of Cu-based complexes. The results showed a value of 6.27 eV for the binding energy of Cu^{2+} and two guanines in DNA [Cu^{2+}]/[guanine] (Xia et al., 2021). In another work, Xia et al. developed a biosensor based on 10-benzyl-2-amino-acridone (BAA) for the colorimetric and photoelectrochemical detection of exosomes. In their study exosomes are captured through hydrophobic interactions and by magnetic beads. To modify the aptamer they used copper oxide nanoparticles. The results of DFT computations predicted Cu^{2+} energy binding to BAA (Xia et al., 2022). Wang et al. demonstrated a conductive elastomer that shows the self-healing features and proposed a microfluidic system. They studied a MXene and graphene oxide conductive network with Al-O bonds in their experiment and investigated the interaction between Al^{3+} and the two nanosheets using DFT computations. They calculated the binding energies of Al^{3+} and functional groups of MXene nanosheets (Wang et al., 2023). In 2023, Zhuang et al. fabricated a novel sandwich-type

biosensor to detect exosomes. They synthesized amino-functionalized Fe_3O_4 nanoparticles and used MXene nanosheets as signal amplifiers. CD63 aptamer connected to Fe_3O_4 nanoprobe can capture exosomes. According to their studies, DFT computations predicted that the Fermi level density of MXene states is affected by the termination of the surface and then consequently affects the electronic conductivity of MXenes. Fig. 4 shows the CD63 protein aptamer-labeled $\text{Fe}_3\text{O}_4\text{-NH}_2$ nanoparticles as the active interface captured exosomes (Zhuang et al., 2023).

Zhang et al. synthesized a hydrophilic bimetallic magnetic MOF composite ($\text{Fe}_3\text{O}_4@\text{UiO-66-NH}_2@\text{PA-Ti}^{4+}$) and used for the isolation of exosomes from biospecimens. According to their report, exosomes can be captured within 5 min. They calculated the particular surface area and pore volume of $\text{Fe}_3\text{O}_4@\text{UiO-66-NH}_2$ using DFT computations (Zhang et al., 2021). Yu et al. proposed a liposome-embedded $\text{Cu}_{2-x}\text{Ag}_x\text{S}$ amplification-based photothermal sensor to do a point-of-care test for the determination of cardiac troponin I. They optimized the structure of $\text{Cu}_{2-x}\text{Ag}_x\text{S}$ and CuS using DFT computations. Their work introduces new insights for sensitive photothermal biosensors and shows that strong near-infrared response of $\text{Cu}_{2-x}\text{Ag}_x\text{S}$ originated from higher carrier concentration (Yu et al., 2022).

5.3. Quantum mechanics/molecular mechanics approach for the applications of exosome biosensors and exosome-mimicking systems

In 1976, Warshel and Levitt introduced QMMM approach to the study of complex molecular systems (Tzeliou et al., 2022; Warshel and Levitt, 1976). Generally, this approach is investigated for the modeling of biomolecular systems, solid-state systems, and inorganic and organometallic structures (Senn and Thiel, 2009). During a chemical process with the forming or breaking of bonds, the electronic structures of molecules can alter. Herein, due to the electronic motion, a quantum mechanical description is required. QMMM is the hybrid quantum mechanics/molecular mechanics approach that can be used on a specific part of the structure where bond breakage and formation happens, e.g.,

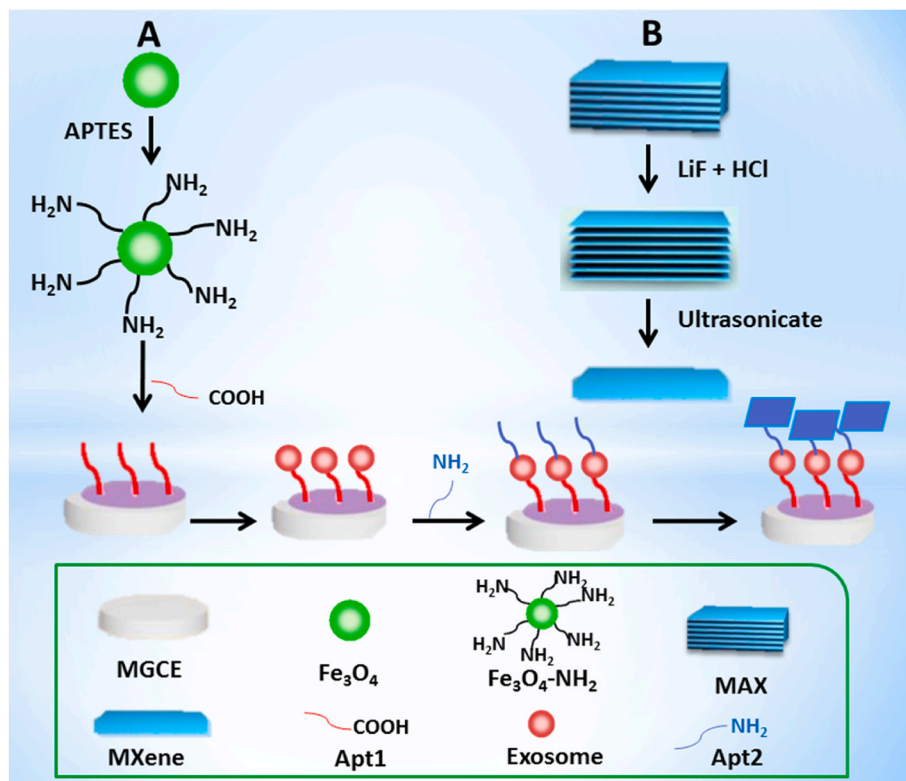


Fig. 4. A schematic of the electrochemical biosensor to recognize of exosome (Zhuang et al., 2023) Copyright 2023, MDPI.

the substrate and catalytic residues (Padhi et al., 2022). In QMMM computations the most crucial task is an impressive interface between QM and MM (Tzeliou et al., 2022). QM methodologies can be precise for small systems. In fact, when the size of the system increases the time of computation sharply increases, and then computation gets expensive. MM methods are much faster than QM computations but with this fact that their calculated energies are not too accurate. QM/MM approach introduces a new methodology that has the good points of QM and MM means it brings the rate of MM and the accuracy of QM (Altoè et al., 2007). Unfortunately, there are no QMMM studies in the field of the exosome-based biosensor or exosome-mimicking systems. Therefore, besides other experimental or computational studies, the need for this method is on the spot and can be introduced as a future work.

5.4. Artificial intelligence

Artificial intelligence (AI) is rapidly influencing our life in various aspects such as access information, how we communicate and even make decision. The investigation of natural cognition, relationships, and behaviors gives insight into model artificially using the algorithms (Sharma, 2024). The AI algorithm is implied in various machines and devices to assist humans. AI in medicine has two branches: virtual and physical. The virtual branch through deep learning information management can do the electronic health records, or help physicians in their treatment decisions. The physical branch is known by robots used by the attending surgeon or assisting the elderly patient (Hamet and Tremblay, 2017). It can be mentioned that AI has even been used for exosome detection. Lu et al. developed a strategy with combining of deep learning algorithm and SERS for label-free profiling of plasma exosomes for early and accurate diagnosis of lung cancer. Their approach showed a significant performance in distinguishing cancer cells from health control samples (Lu et al., 2024). The same research has been carried out by Xie et al. Their strategy demonstrates the cancer cell-derived exosome with 100% purification accuracy for patients with different breast cancer subtypes (Xie et al., 2022).

6. Conclusion

The early detection and effective treatment of cancers pose significant challenges, and traditional diagnostic methods often fall short due to the impracticality of repeated screenings and limited accessibility to large populations. To tackle these challenges, cancer-derived exosomes are ideal candidates as next-generation biomarkers; on the other hand, biosensors, offer a straightforward implementation for the detection of these exosome-based biomarkers, leveraging their sensitivity and selectivity towards various structures. While the design and optimization of biosensors present challenges, computational methods provide researchers with the ability to predict the behavior of biomolecules, offering a deeper understanding of the design and optimization processes. This review highlights the use of molecular docking, molecular dynamics simulation, and density functional theory computation, in the development of exosome-based biosensors.

It also emphasizes the relatively scarce number of computational works in this field, and notably, the limited application of the Quantum Mechanics/Molecular Mechanics approach, besides other experimental or computational studies suggesting it as a motivation for future research.

CRediT authorship contribution statement

Maryam Atabay: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Fatih Inci:** Writing – review & editing, Visualization, Validation, Project administration, Investigation, Funding acquisition, Conceptualization. **Yeşeren Saylan:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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