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REVIEW ARTICLE

Molecular Docking: Principles, Advances, and Its Applications in Drug Discovery

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Abstract: Molecular docking is a structure-based computational method that generates the binding pose and affinity between ligands and targets. There are many powerful docking programs. However, there is no single program that is suitable for every system. Hence, an appropriate program is chosen based on availability, need, and computer capacity. Molecular docking has clear steps that should be followed carefully to get a good result.

Molecular docking has many applications at various stages in drug discovery. Although it has various application areas, it is commonly applied in virtual screening and drug repurposing. As a result, it is playing a substantial role in the endeavor to discover a potent drug against COVID-19. There are also approved drugs in the pharmaceutical market that are developed through the use of molecular docking. As the accessible data is increasing and the method is advancing with the contribution of the latest computational developments, its use in drug discovery is also increasing.

Molecular docking has played a crucial role in making drug discovery faster, cheaper, and more effective. More advances in docking algorithms, integration with other computational methods, and the introduction of new approaches are expected. Thus, more applications that will make drug discovery easier are expected.

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1. INTRODUCTION

Computer-aided drug design (CADD) is an area that consists of many computational strategies for the discovery, design, and development of novel therapeutic agents. CADD has a crucial role in improving active ligands, discovering novel drugs and understanding biological processes at a molecular level [1]. Furthermore, the application areas of CADD methods are widening with the increase in biological and chemical data, increase in data storage capacity, increase in identified drug targets, and advance in data processing capacity [2].

CADD methods enable rapid, economic, and more efficient drug discovery and development [3]. The drug development process includes drug discovery, preclinical studies, clinical phase studies, and registration. This is an expensive process that takes more than 10 years on average [4]. CADD has applications mainly in the drug discovery phase of this process [5]. CADD provides the advantage of filtering smaller series of compounds expected to be active from large compound libraries and therefore guiding to find of the lead compounds, optimization of the lead compound, and designing novel compounds in the drug discovery [6, 7]. In addition to this, CADD methods can sometimes replace *in vivo* models and lead to the formation of high-quality datasets [8]. There are several approved drugs in the market that are developed through CADD. For example, the anti-HIVs raltegravir, saquinavir, indinavir, and ritonavir, the anti-influenza oseltamivir, the antihypertensive captopril, the carbonic anhydrase inhibitor dorzolamide and the neuraminidase inhibitor zanamivir are developed by using CADD methods [9].

Based on the type of data available, computer-aided drug design methods can be categorized as target-based and ligand-based drug design methods [10]. In the target-based drug design, the aim is to design potential active compounds by using target structures. Although docking is a typical example of this approach, molecular dynamics and binding site estimation methods can also be evaluated in this class [11, 12]. Homology modeling can be employed at this approach's early stages in case the protein's three-dimensional structure hasn't been determined yet [13]. In the ligand-based drug design, the aim is to interpret the structure of the target by using the structure of active ligands. Pharmacophore modeling and QSAR (quantitative structure-activity relationships) are examples of this approach [14]. QSAR models effectively estimate experimental activities based on molecular de-

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scriptors [15]. To increase the performance of CADD, it is important to use both approaches together in a way that complements each other. This is also known as the hybrid approach [1]. For example, molecular dynamics is utilized in both methods for the discovery of novel drug candidates [13]. There is also a method named *de novo* molecular design that is used to design novel chemical entities which satisfy a desired molecular profile [16]. This method gives the opportunity to generate novel molecular structures in the abscence of a starting template [17]. In this study, molecular docking, which is one of the target-based drug design methods, is reviewed.

Molecular docking is a structure-based computational method that generates the binding mode and affinity between ligands and targets by predicting their interactions [18, 19]. There are several docking tools used for this purpose. AutoDock, AutoDock Vina, GOLD, Glide, MOE, ICM, and FlexX are amongst the popular software in use [20]. Many of them are powerful docking tools, but there is no sole software suitable for every system. Thus, users should choose their preferable software based on availability, their needs, and their computer capacity. It is also possible to use more than one software in a way that increases the quality of the output [21].

Molecular docking has various applications in the drug discovery and design process. In the early years of its application, it was mainly used to investigate the molecular interactions between ligands and targets [22]. Nowadays, it supports wider and more diverse areas of drug discovery [23]. It has applications in virtual screening, target fishing, drug side effect prediction, polypharmacology, and drug repurposing. With the involvement of state-of-the-art computational approaches like artificial intelligence, there is an advance in docking algorithms. The integration of molecular docking with other approaches, such as ligand-based methods, is also underway. Moreover, there is a significant increase in open-source biological and chemical data. All these are contributing to the advance in molecular docking. With the advances in molecular docking, its application in drug discovery is rapidly increasing [11, 24].

Molecular docking has substantially brought anti-HIV, anticancer, and various other drugs to the pharmaceutical market [25]. A typical example of the successful application of molecular docking is the design of rilpivirine [26]. The molecular modeling studies that led to the discovery of rilpivirine involved the docking of diarylpyrimidine ligands into the reverse transcriptase binding site. The computational assessment followed by the experimental evaluation resulted in the approval of rilpivirine against HIV [27]. Similarly, molecular docking guided the design of betrixaban. A lead compound with improved potency was discovered [28]. The binding mode of the lead compound was elucidated by docking using the GOLD program. Based on docking and other computational observations, further modifications that led to the development of betrixaban were performed [29]. In another example, molecular docking was used in the discovery of the neuraminidase inhibitor zanamivir. Molecular docking was used to analyze the active site of neuraminidase and its interactions with its new inhibitors. The interaction of zanamivir with neuraminidase was elucidated [30]. After the computational results were confirmed by the *in vivo* tests, the drug was approved [25]. Flexible molecular docking was also used in the discovery and development of drugs [31]. For instance, it was utilized in the discovery of vaborbactam. ICM docking was used in the design of lead β-lactamase enzyme inhibitors with better activity. The lead molecule was evaluated with computational and experimental methods. Finally, vaborbactam was developed [32].

There is a great effort worldwide to discover an effective drug to combat the global pandemic, COVID-19 (Coronavirus disease 2019) [33]. The available literature shows the discovery of new potent molecules against novel coronavirus is still at its early stage [34]. Thus, virtual screening and drug repurposing are recommended as the fastest options for the discovery of a potent drug against the novel coronavirus [35, 36]. As molecular docking has been commonly used in virtual screening and drug repurposing, it can play a substantial role in discovering a potent drug against the novel coronavirus [37]. Therefore, it has been applied and recommended to be used in the endeavor to discover promising drugs against the coronavirus using computational methods [38].

Molecular docking has established applications at various stages of the drug discovery process. With the contribution of the latest computational advances, it is expected to have more applications [25]. Thus, updated information about molecular docking is in need. This review is aimed at meeting this demand in academia and the pharmaceutical industry. In this work, the basic principles of molecular docking, including its steps are presented. Current applications of molecular docking in drug discovery are explained with examples. Furthermore, the challenges and advances in molecular docking are summarized.

2. PRINCIPLES OF MOLECULAR DOCKING

Docking is a method based on the examination of the fitting of the designed compounds to target cavities and their interactions with the residues [39]. In the computational drug discovery process, docking is generally undertaken between small molecules and macromolecules, as in protein-ligand docking. This type of docking is known as molecular docking. Over the last few years, docking has also been performed between two macromolecules, as in protein-protein docking [40].

The basis for the majority of the docking programs is molecular mechanics, which explains polyatomic systems using classical physics. Experimental parameters are used to reduce the deviation between the experimental data and molecular mechanics. Due to the limitations of the experimental methods, mathematical equations are converted into parameters using quantum mechanics semiempirical and *ab initio* theoretical calculations [39]. In this regard, it is a set of equations with different parameters that aim to define molecular force field systems, which are based on potential energy, torsional properties, the geometry of the bond, electrostatic terms, and Lenard-Jones potential. AMBER, CHARMM, GROMOS, OPLS-AA, and UFF have known examples of force fields [41].

In the 1980s, molecular modeling was performed using force fields. In the continuation of these methods, modeling of molecular processes like the binding of ligands to their target proteins was undertaken. Two main methods are built for this purpose: Rigid body and flexible docking [10]. In rigid body docking, ligands and targets are considered two different bodies that recognize each other according to their shape and size. In flexible docking, protein-ligand recognition occurs by considering the effect of the two structures on each other [42]. In early practices, a rigid ligand was docked into a rigid target. With the advances in computing power, new efficient computational methods that enable the docking of flexible ligands into rigid targets are introduced. As the targets are also flexible at physiological conditions, their conformational changes are expected to be addressed. Otherwise, ligands that could bind to a target could give a mistaken interaction in computational analysis. This is addressed by introducing target flexibility in molecular docking. Addressing the targeting flexibility requires additional computational resources. Approximate methods that make it practical have been introduced [43].

There are many servers and programs that are used in molecular docking. In each program, various force fields and algorithms are used for pose prediction, refinement, and generation of the target-ligand interactions (Table **1**) [44]. Although there are many powerful docking programs, it is good to remember that none of the docking algorithms in use are suitable for every system. It is recommended to use more than one program [21].

2.1. General Recommendations and Guidelines for Molecular Docking

2.1.1. Hardware and Software Requirements for Molecular Docking

As ligand docking and computing are performed in a few minutes, docking computations are not considered intensive processing unit (CPU). Currently, almost every personal computer (PC) is capable of running small docking works (500-1000 compounds) in an acceptable time [61]. However, in the virtual screening of public databases using docking-based methods, the number of molecules could rise rapidly (10^6 compounds). This requires more data processors to finish the process in a reasonable time. Generally, GPU data processing is more efficient and attractive for intensive processing than CPU-based computations [40].

2.1.2. Program Selection in Molecular Docking

There are many docking methods and approaches (Table **1**). Among these methods, for beginners, easily accessible academic or free software are preferable. Some of the docking programs are not designed to run on Windows [62]. In such cases, beginners can start with Linux and overcome the problem. In addition to this, Windows-friendly programs such as AutoDock, Vina, and LeDock can be preferred [40].

2.2. Steps of Molecular Docking

The molecular docking process consists of target protein determination and preparation, ligand preparation, determination of the type of docking to be used, selection of the best docking scoring function, and validation (Fig. **1**) [40, 63].

2.2.1. Target Protein Determination and Preparation

The properties of the selected protein structure affect docking results [64]. With the development of X-ray crystallography, NMR, Cryo-EM, and similar structure determination methods, the number of proteins with known three-dimensional (3D) structures is rapidly increasing and they are accessible to the public in databases like the protein data bank (PDB) [65]. The first step of docking is retrieving the 3D structure of the protein, preferably bound by a ligand, from the PDB. Using 3D structures with high resolution (< 2Å) or structures bound by a high-affinity ligand is suggested. The situation may be different for a few proteins [23]. In such cases, using structures that structural studies have previously investigated might be appropriate [66]. Furthermore, if the 3D structure of the protein hasn't been determined yet and is thus not available in the PDB, it should be built by homology modeling [67].

Molecular docking needs the specification of some parameters. The PDB files often have deficient information and therefore, they need to be corrected [68]. In the preparation of the protein, hydrogens must be added, water should be removed, charges must be assigned, and energy minimization should be undertaken. There are several preparation modules that fix common problems of PDB files [69].

Parameterization methods used vary depending on the software [70]. AutoDock and SwissDock utilize an in-program force field, whereas MOE uses AMBER and LeDock uses CHARMM charges and atomic species. Therefore, it is important to employ the same preparation protocol in all docking procedures to compare the respective docking results [71].

2.2.2. Preparation of Ligand

The structure of the ligands is drawn with programs like ChemDraw [72] or is downloaded from chemical libraries or databases like PubChem [73] and ZINC [74]. Before using these structures in docking, energy minimization should be undertaken [75].

It is recommended to visually examine the results of the preparations of the target and ligand. Because some preparation methods can lead to mistakes in molecular descriptions, such as incorrect connection, missing bonds, and abnormal geometries. These errors often occur during the conversion of one molecular format to another. Hence, it spreads easily [71].

After preparing the target and ligand, the binding site should be determined and limited. It is possible to do this step either by the specification of the coordinates manually or by utilizing the coordinates of a ligand attached to the protein. There are also programs that are used to calculate the probable binding site [76]. The grid makes mapping the binding area, which will be the center of docking calculations. The grid can be thought of as a box with known dimensions split into small squares in which the probe atoms describe the contour of a possible interaction. Resolution and size of the grid affect docking results [46].

Table 1. Molecular docking programs.

Program	Availability	Properties
AutoDock [45]	Free	Rigid body-flexible docking. It is used with Autodock tools. Calculation of the grid maps is automatic.
AutoDock Vina [46]	Free	Rigid body-flexible docking. It applies recurring local search global optimization. It is faster than AutoDock. It provides improved binding affinity prediction with a new scoring function.
Dock [47]	Academic	Flexible docking. It is widely applied to flexible targets and flexible ligands.
LeDock [48]	Academic	Flexible docking. Since it gives results fastly with high accuracy, its use in virtual screening is recommended.
FlexX [49]	Commercial	Rigid body-flexible docking. It can be utilized in virtual screening.
Glide [50]	Commercial	Ligands are flexible in this docking. To decrease the software search range, it uses information about the area. It has XP (extra precision), SP (standard precision), and highly efficient virtual screening modes.
GOLD [51]	Commercial	Flexible docking. The evaluation of its accuracy and reliability appeared to give good results.
Plants [52]	Academic	It has a good balance between usage and efficiency. It allows calculating water exchange.
ICM [53]	Commercial	It gives the facility of both ligand-protein and protein-protein docking. It provides an ICM-Pro interface that makes the docking process easy.
MOE [54]	Commercial	It has a good interface and intuitive aspect. It also consists of other tools that are used in protein and ligand preparation.
Surflex [55]	Commercial	For predocking minimization and post docking optimization, it uses procedures. It makes use of morphologic similarity functions and fast pose production techniques.
LibDock [56]	Academic	LibDock depends on the matching of the polar and apolar binding site features of the target-ligand complex. As it is driven by matching features rather than a molecular mechanics force field score, its performance attracts interest.
CDOCKER [57]	Free	CDOCKER (CHARMM based DOCKER) provides the advantages of full ligand flexibility, CHARMM force field, and reasonable computation time. Flexible docking.
Fitted [58]	Free	Fitted can deal with both macromolecule flexibility and the presence of bridging water molecules.
Molegro [59]	Free	The program Molegro Virtual Docker (MVD) has four search algorithms and four native scoring functions. MVD provides the opportunity of performing detailed statistical analysis of docking results when it is integrated with other programs.
Fred/Hybrid [60]	Commercial	Fred uses the target structure solely to pose and score ligands. On the other hand, Hybrid uses both the target and ligand structures to pose and score ligands. Hybrid has the ability to use multiple conformations of the target.

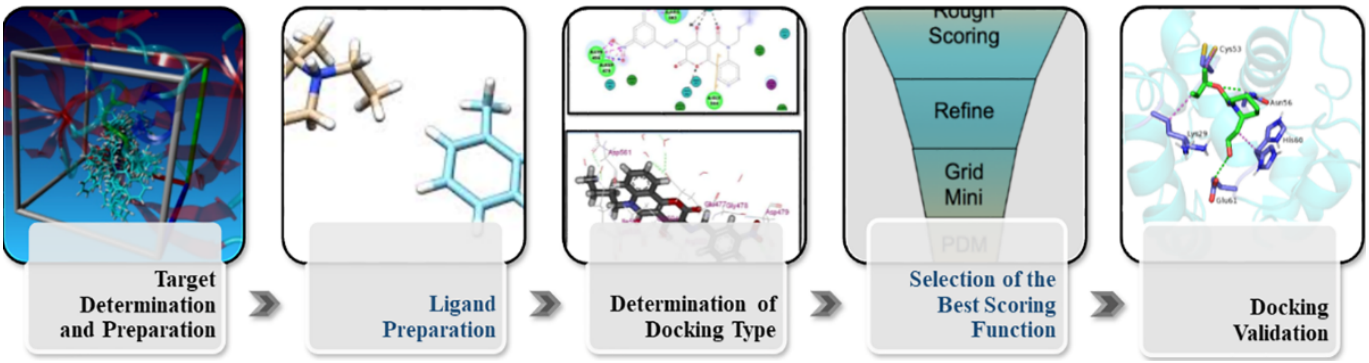

Fig. (1). Steps of molecular docking. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

2.2.3. Determination of Docking Type

The choice of docking type to be used depends on the needs of the researcher. If docking of several molecules at the binding site of a protein at a specific pH, water, and solubility is desired, flexible docking programs may be preferred. However, if many more compounds (in thousands) are to be scanned from databases, flexible docking methods may be a bad option unless there is a high processor and a fast computer. Therefore, the user can choose different docking methods according to the computer's capacity and the target's properties [77].

2.2.4. Selection of the Best Docking Scoring Function

The best docking scoring function is selected depending on the stability of the ligand-protein complex. It is difficult to choose a suitable scoring function that gives a correct binding pattern and the possible ligand. Theoretically, the lower the binding free energy (ΔG) of a protein-ligand complex, the more stable the complex is [78, 79].

Docking score is computed by various programs to identify and rank many poses of a ligand in a reasonable time [80]. Scoring functions should be able to differentiate binders from nonbinders clearly. In addition to this, it should be able to discriminate between correct and incorrect binding modes of a ligand with high accuracy and in a reasonable time [81]. Scoring functions are classified into three main categories: Empirical, force field, and knowledge-based. In empirical scoring functions, the free energy of binding is calculated by adding hydrogen bonding, Van der Waals interactions, electrostatics, hydrophobic interactions, and the conformational free energy released when a ligand binds. In the force field method, force field energy is computed using molecular mechanics force fields similar to those used in CHARMM and AMBER. This energy includes internal energies, coulombic interactions, including Van der Waals interactions and hydrogen bonding. The entropy and solvent energies are calculated separately. Knowledge-based scoring functions are calculated by converting the frequencies of ligand-protein atom interaction pairs into free energies using Boltzmann distributions [15]. A single scoring function is not perfect. Hence, it is possible to combine different scoring functions to improve calculations with a single scoring function. This method is known as consensus scoring [80].

2.2.5. Docking Validation

Like any other technique, the docking process should also be validated. The docking results are validated by redocking of reference ligands with targets and comparing the RMSD (root mean square deviation) values, binding pose, binding affinity, and coverage of the estimated bindings with previously acquired results. If the ligand and target structures are complex, it is recommended to carry out molecular dynamics studies. Molecular dynamics simulations can be utilized to optimize the target before and after docking and to provide flexibility, fix the complex after docking, calculate the binding free energy including the solvent effect, and ensure the correct sequence of possible ligands [82]. At the end of the process, the binding pose, binding residues and binding energies of the ligands are revealed (Fig. **2**).

3. APPLICATIONS OF MOLECULAR DOCKING IN DRUG DISCOVERY

With advances in docking algorithms, an increase in open-access information on ligands and targets, the applications of molecular docking in drug discovery are rapidly increasing [83]. In the early years, it was mainly used in the investigation of the molecular interactions between ligands and targets (15). However, these days the application scope is wider and there is somewhat a shift in the application area. Molecular docking has applications in virtual screening, target discovery and profiling, drug side effect prediction, polypharmacology, and drug repurposing (Fig. **3**) [24].

3.1. Virtual Screening

Virtual screening is used to find hits and lead compounds from molecular databases according to scoring functions [84]. The applications of docking in virtual screening have increased with the combination of the method with other

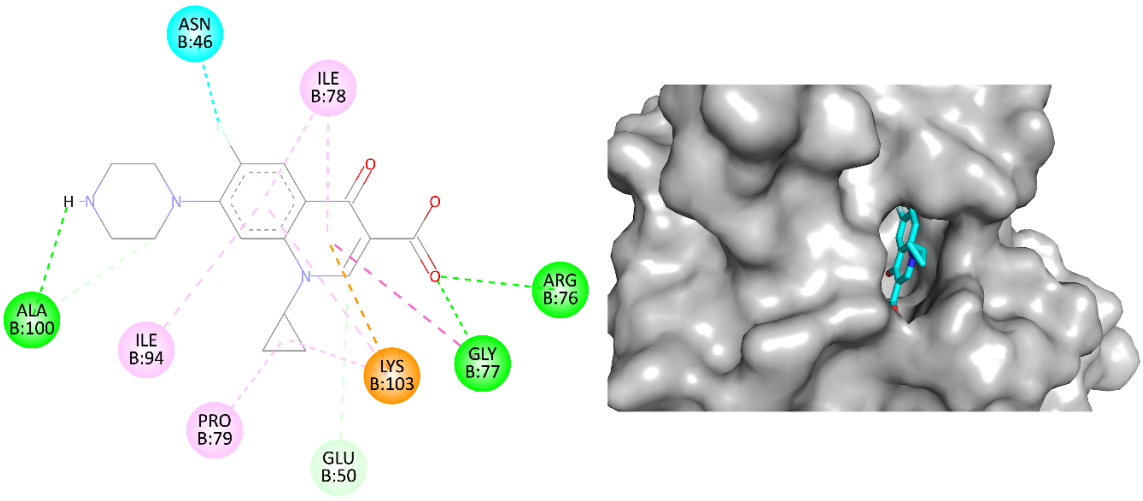

Fig. (2). Binding residue points and binding pose of ciprofloxacin inside the binding site of DNA gyrase B. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

new applications. For example, the combination of molecular dynamics and free energy binding estimation methods with docking has improved virtual screening [85].

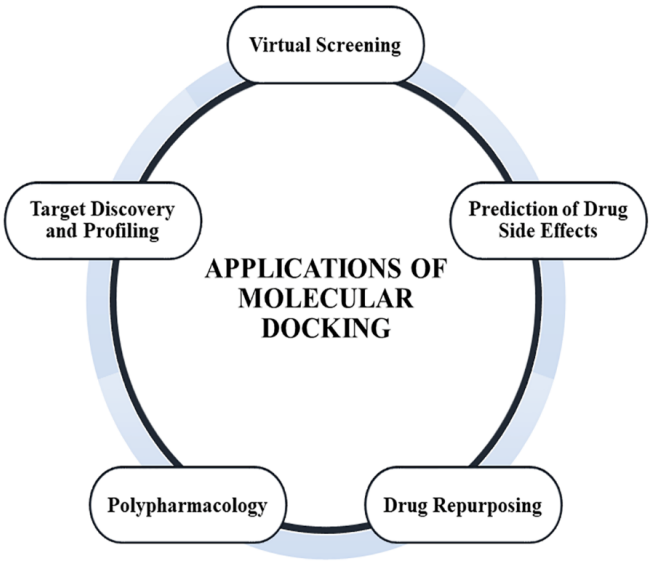

Fig. (3). Applications of molecular docking in drug discovery. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

These days there is a great effort worldwide to discover a promising drug against the global pandemic, COVID-19. There are efforts to discover drugs using SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) targets such as the structural spike (S) protein, envelope (E) protein, membrane (M) protein, nucleocapsid (N) protein, and non-structural proteins (Nsps) like the main protease (also called 3C-like protease ($3CL^{pro}$, nsp5)), papain-like protease (PL^{pro}, nsp3), RNA-dependent RNA polymerase (RdRp, nsp12), nsp15 endoribonuclease, nsp16 (2′-O-methyltransferase) and nsp13 helicase. Host-based targets like angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), furin, and cathepsin are also used in this effort [86]. Molecular docking has been used together with other methods to support this effort [87]. For instance, researchers performed an *in silico* screening of phytochemicals and revealed that some of them could be effective against SARS-CoV-2. Selected 154 herbal chemicals were docked to five therapeutic protein targets of SARS-CoV-2 (proteases, PL^{pro}, SGp-RBD, RdRp, and ACE2) by using AutoDock Vina. Using the docking score, the best 20 herbal chemicals for each protein were screened for further investigation. By using further computational analysis methods, 7 herbal chemicals were proposed as potential SARS-CoV-2 inhibitors for further *in vitro* and preclinical tests [88]. Similarly, 2000 molecules from the Selleck database of natural compounds were screened by using ensemble docking against the main protease (M^{pro}). The compounds that exhibited better binding were filtered further by using Molecular Dynamics (MD) simulations. Then, 11 natural compounds that were found to bind to M^{pro} protease well were purchased and tested *in vitro*. Finally, five promising M^{pro} protease inhibitor natural compounds were determined [89].

In another similar work, a structural study was performed to identify promising drug candidates to fight against COVID-19. In this work, virtual screening together with molecular docking was performed to look for potential inhibitors of the M^{pro} of SARS-CoV-2. Virtual screening was done by using the Glide docking module. First, 50 molecules from 2100 FDA-approved drugs in the ZINC database and 20 molecules from 400 natural products in the Spec database were screened based on their docking score, glide energy, and hydrogen bond interactions. Then, with XP glide docking and MD simulations, two compounds were suggested for further experimental tests [90]. Similarly, hits from two *in silico* screening studies were utilized in a wet-lab study to identify potential M^{pro} inhibitors. The REAL Space or ZINC databases were screening by ranking the molecules using docking parameters [91, 92]. The promising compounds were synthesized and assayed for their ability to inhibit the activity of M^{pro}. Five compounds were found to inhibit the enzyme *in vitro* [93].

3.2. Target Discovery and Profiling

Reverse docking allows the prediction of the biological target of the respective molecule. As a result, it is a valuable approach in computational target discovery and profiling [94]. There are many docking approaches and algorithms for reverse screening of a ligand against protein structure libraries and evaluation of its binding affinities. However, the implementation of these methods needs a convenient target library [95]. There are several databases available for reverse docking screening. PDTD (potential drug target database) is a good example of familiar databases used in this area [96]. In addition to this, target libraries can be prepared manually from databases such as PDB and TTD (therapeutic target database) [97]. Reverse docking tools and web servers like TarFisDock [98], idTarget [99], INVDOCK [100], DockoMatic [101] and SePreSA [102] are available for researchers.

In reverse docking screening, for a ligand, probable targets can be ranked by using the scoring functions used in the programs [50]. For example, research using reverse docking therapeutic mechanisms of astragaloside IV was investigated. In this study, all signaling pathways thought to be implicated in the therapeutic actions of all cardiovascular disease drugs approved by the FDA were considered. At the end of the study, 39 putative targets were identified, and three of them (CN, ACE, and JNK) were experimentally validated [103].

3.3. Prediction of Drug Side Effects

Early detection of adverse drug effects is of great importance in the drug discovery process. Drug candidates have been reported to fail clinical trials mainly due to adverse effects from unanticipated off-target interactions [104]. There are many computational approaches to support this work. However, most model exercises require sufficient bioactivity data or previously reported side effects [105]. To predict side effects, molecular docking requires only structural information about the target. It is, therefore an important approach in predicting potential side effects of molecules in the early phases without having detailed information about the drug and bioactivity records. For example, re-

searchers conducted a reverse docking screening with torcetrapib, a cholesteryl ester transfer protein inhibitor, to investigate the increase in mortality and cardiac events associated with the side effects of the drug. Torcetrapib was docked into a set of protein targets based on the enriched signaling pathway. The results demonstrated that platelet-derived growth factor receptor (PDGFR), hepatocyte growth factor receptor (HGFR), IL-2 Receptor, and ErbB1 tyrosine kinase might be the potential off-targets [106]. Databases that facilitate the identification of drug side effects have been developed. However, good performance in these predictions directly depends on the information in the databases. SIDER (side effect resource) is one of the databases known in this area [107]. Furthermore, by combining docking with machine learning (ML) and statistical approaches, advanced screening methods, which also make drug side effect predictions more advanced, were developed [108].

3.4. Polypharmacology

Polypharmacology expresses the identification of ligands that interact with targets with a selected series of therapeutic values. The pharmaceutical industry has concentrated on the development of immensely selective drugs to avoid possible side effects [109]. However, the high failure rate experienced in the final phases of clinical tests as a result of lack of therapeutic activity has led new drug designs to shift to polypharmacology [110]. In this regard, molecular docking provides a valuable opportunity as it permits the identification of chemical structures that interact effectively with related targets simultaneously. It is difficult to design multitarget ligands for rational reasons. Furthermore, the choice of protein structures to be utilized for docking can greatly influence the outcome of the design. This is particularly the case when working with targets with remote binding sites [111]. Docking is currently used in combination with other *in silico* methods by considering the challenge of multitarget drug design. Especially, several studies that comprise the determination of multitarget ligands by applying docking screening together with pharmacophore modeling have been reported [112]. The determination of the first binary inhibitor of Hsp90/B-Raf is an example. In this study, it has been shown that substructure prefiltering and pharmacophore-led docking can effectively be combined to look for polypharmacological ligands whose structures interact with different targets. In another recent study, the potential of the cationic pentapeptide Glu-Gln-Arg-Pro-Arg was assessed for its potential role as an anticancer and anti-SARS-CoV-2. The binding affinity of the peptide to integrins, M^{pro}, S protein, and ACE2 was evaluated using molecular docking [113]. Polypharmacology workflows that combine docking with other *in silico* methods were also followed [114].

There are docking-based web tools and platforms used to investigate polypharmacology and determine the ligands' multitarget activities. CANDO (computational analysis of novel drug opportunities) [115] is an example for platforms and DRAR-CPI (drug repositioning and adverse reactions *via* chemical protein interactome) [116] is an example for web servers.

3.5. Drug Repurposing

Drug repurposing is an established drug discovery way that provides the opportunity to identify new therapeutic applications for approved drugs, drug candidates under evaluation, natural products, or generally presynthesized ligands [117]. Considering the wealth of information available in public databases on ligands, targets, and diseases, efforts to increase the application of the discovery strategies based on *in silico* repurposing have increased over the last decades. *In silico* repurposing methods have been shown to offer valuable new opportunities in drug discovery and development [118].

In this regard, molecular docking is among the most widely used computational methods used for repurposing ligands toward new therapeutic targets [119]. Docking lets virtual screening of databases of approved drugs, phytochemicals, or presynthesized compounds to the target of interest in a reasonable time [95].

Recently, there are many studies that focus on repurposing existing drugs to combat COVID-19 [120]. For example, researchers searched for commercially available drugs to repurpose them against SARS-CoV-2 using *in silico* approaches. In this work, structure-based screening of approved drugs against M^{pro} and the serine protease TMPRSS2 of the novel coronavirus. Homology modeling was used to generate the 3D structure of TMPRSS2. The structure-based screening was performed by AutoDock Vina, and the resulting top-ranked hits were selected. With further molecular docking using AutoDock 4.2, the best hits based on docking score were screened. Then, with ADMET profile and drug-likeness predictions, four approved drugs (talampicillin, lurasidone, rubitecan, and loprazolam) from the drug library were found to be potential inhibitors of M^{pro} and TMPRSS2 of the novel coronavirus. The stability of the complexes was also checked by MD simulations [121].

In another study, researchers identified potential inhibitors of M^{pro} of the novel coronavirus using *in silico* drug repurposing. Molecular docking calculations were carried out using AutoDock 4.2 to select top-ranking drugs from the DrugBank database. After the top-ranked approved drugs from the database were filtered, 35 drugs with docking scores of lower than -11.0 kcal/mol were picked for further investigations. Then with MD simulations followed by MM-GBSA (molecular mechanics–generalized Born surface area) binding energy calculation, DB02388 and cobicistat (DB09065) were found to be potential inhibitors for M^{pro} of the novel coronavirus [122]. Similarly, mechanistic investigation of the interaction of teicoplanin with M^{Pro} has been done by molecular docking and molecular dynamics to repurpose it against SARS-CoV-2 [123]. Similarly, inhibitors of its homolog, Hepatitis C Virus (HCV) protease, were investigated to repurpose them as M^{pro} inhibitors. 20 direct acting antivirals of HCV were docked against M^{pro} and six of them were found to be promising inhibitors [124]. In another similar work, Remdesivir was found to be one of the hits for M^{pro} inhibitors [125]. The available literature shows research in discovering new molecules against novel coronavirus is still in its infancy. Thus, virtual screening and drug repurposing of the available databases are the fastest options for the

discovery of potent drugs against the novel Coronavirus [35].

Based on these promising results, it is possible to say that docking is a valuable approach in drug repurposing. Especially, when it is combined with other computational approaches, such as ligand-based methods, its value increases [24].

4. CURRENT STATUS OF MOLECULAR DOCKING

Molecular docking is broadly used in the academia and pharmaceutical industry [11]. The wide scope of its applications, exemplified in the previous sections, demonstrates the opportunity it provides for drug discovery. As is expected, research works in molecular docking have been increasing. Thus, the number of published articles in this area is rapidly increasing (Fig. **4**). To investigate the extensive usage of molecular docking over the last two decades, the number of documents available in publication databases has been found. For this purpose, Scopus, PubMed, and ScienceDirect search engines were used. These search engines were preferred since they were found to offer good search tools and demonstrated satisfactory performance [126]. In each of them, published documents were searched by using 'molecular docking' as a keyword. After extracting the number of documents by year in the three engines, the average number was calculated for each year starting from 2000. By using these data, the graph of publications in the last two decades was drawn (Fig. **4**).

The results demonstrated that the number of publications generated had nearly doubled every five years (Fig. **4**). This is in line with other similar studies conducted before. Currently, as illustrated by the publications, molecular docking is widely used [20, 127].

Although many powerful programs are used in molecular docking, there is no single program suitable for every system. Consequently, users choose their preference depending on the availability of the program, their needs, and their computer capacity. They might also utilize more than one program [21]. Therefore, molecular docking programs are expected to have different popularity.

In this work, the relative popularity of selected docking tools was also investigated. The total number of published documents in Scopus, PubMed, and ScienceDirect search engines was extracted. To find the total number of documents, 'docking' and the respective docking tools were used as a keyword together. After the calculation of the average total publication until 2020, the relative popularity graph was drawn (Fig. **5**). AutoDock was found to be the most popular docking tool. Furthermore, GOLD and Glide were found to be popular among commercial docking tools (Fig. **5**). Previously reported studies also gave a similar popularity degree [20, 127]. There has also been a considerable increase in the popularity of AutoDock Vina in the last few years [128].

5. ADVANCES IN MOLECULAR DOCKING

The opportunities provided by molecular docking in the drug discovery process are well known. However, intrinsic factors limit the prediction performance of docking [129]. Thus, although it is essentially a stand-alone method in drug design, these days it is used in combination with other computational methods like ligand-based approaches, the rest of structure-based approaches, quantum mechanics, machine learning, and artificial intelligence (AI). This paves the way to overcome some of the most important shortcomings of docking [24].

5.1. Contribution of Ligant-Based Approaches

Ligand-based approaches have been used to identify appropriate target structures for docking-based screening. The ability of docking to differentiate active compounds from inactive ones may have a high dependence on the 3D structure of the target used and the degree of similarity of the selected ligands by screening against those compounds co-crystallized in the target structure. Similarly, ligand-based approaches have been utilized to increase the predictive potential of docking screening [130]. For example, it can con-

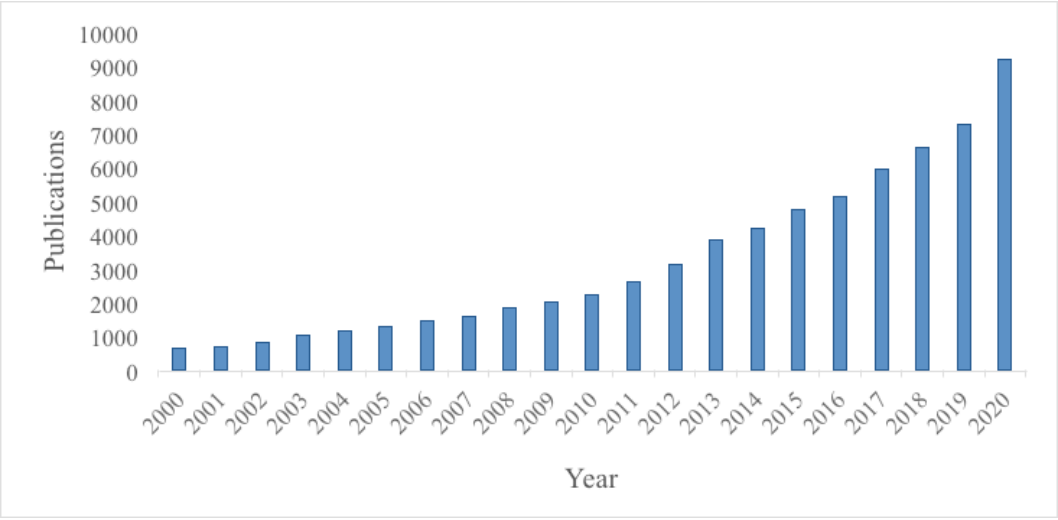

Fig. (4). Publications in molecular docking. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

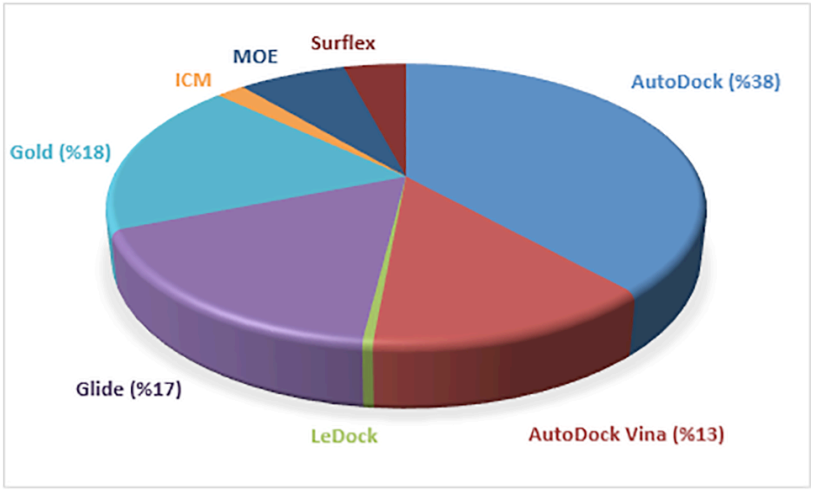

Fig. (5). The relative popularity of docking tools. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

tribute to the evaluation of the 3D structure resemblance between the binding pattern estimated by docking and the experimentally detected binding pattern of the co-crystallized ligand to the target structure. However, it shouldn't be forgotten that the possibility of using ligand-based approaches in combination with docking applies only to targets with a minimum of one reported co-crystallized ligand [131].

5.2. Contribution of Structure-Based Approaches

Structure-based approaches, especially molecular dynamics (MD) and binding free energy prediction, have been broadly used in combination with docking to improve virtual screening [132]. In this regard, MD is used to measure amino acid flexibility in the binding site and to investigate greater structural changes with potential accessibility to a given protein. Therefore, it is an efficient tool for the determination of target structures for docking and evaluation of the stability of the predicted complex [10]. The opportunities provided by MD *in silico* screening, especially, address flexible targets with few elucidated 3D structures. The contribution of binding free energy estimation to the improvement of virtual screening has also been investigated. The output of currently used docking algorithms might be affected by poor structural sampling. They can also give incorrect binding energy predictions. Many approaches, such as BEAR (binding estimation after refinement), MM-PBSA (molecular mechanics–Poisson Boltzmann surface area), and MM-GBSA methods, have been taken to address these issues [133]. These approaches have also been shown to improve virtual screening and docking results [134].

5.3. Contribution of Quantum Mechanics

The contribution of Quantum Mechanics (QM) in improving the prediction of binding free energy by molecular docking is acknowledged [135]. The priority of molecular mechanics (MM) scoring functions is speed rather than accuracy. Therefore, the reliability of predicting the free energy of protein-ligand binding interactions is limited [136]. QM calculations can be used to improve the prediction of binding affinities, including re-scoring in docking [137]. The application of QM calculations in docking rescoring brings a better electrostatic interaction description and interaction energy. QM can also play its role in dealing with ionization and tautomerism. Thus, QM-based scoring functions provide a better correlation of calculated and experimental ligand affinities than the classical MM. This in turn, improves its role in lead optimization [136].

5.4. Contribution of Machine Learning

Scoring and ranking candidate molecules by the calculation of binding affinity is a very challenging issue in molecular docking. Classical scoring functions need to simplify and generalize several features of receptor-ligand interactions to maintain efficiency, approachability, and accessibility [77]. In addition, classical scoring functions use linear regression models, parametrically controlled learning methods that take a predetermined functional form. Here, parametric methods convert the input variables to the output forms with a predefined function and adjust them in a theory-inspired manner during the creation of the scoring function. This rigid scheme often results in unadaptable scores that do not capture the intrinsic nonlinearities of the data. Therefore, they show low performance in cases that are not considered in their formulations [138].

Machine learning algorithms can be used to improve or replace predetermined function forms used in binding affinity prediction in classical scoring. These have also been used to identify binders/non-binders in virtual screening [139]. Machine learning, nonparametric learning, does not take the form of predetermined functions. Instead, outputs are extracted from the input data. It can give a continuous output as in nonlinear regression. This in turn allows for diverse and accurate scoring. Random forest (RF)-Score is one of the first machine learning scoring functions that outperform classical scoring functions. In addition, logistic regression and support vector machines (SVM) were used to improve docking-based binding affinity predictions [138, 140, 141].

5.5. Contribution of Artificial Intelligence

Artificial intelligence (AI) allows easy use of the ever-growing open-access information sources in chemical, structural, and biological activity databases. This increases the accuracy of binding affinity estimations [142]. In this context, deep learning neural networks have been used in pose generation and scoring [143]. The convolutional neural network has been investigated in molecular docking by designating protein-ligand complexes as 3D cages. Deep learning scoring functions have produced comparable and even superior results to machine learning and other non-neural network algorithms [144, 145]. Machine learning might also be treated as a member of this class. AI-based ML learns from the properties of the available data and then makes predictions on blind data [146]. These approaches might not be preferable to newly discovered therapeutic targets that have not been thoroughly investigated yet and thus chemical, structural, and bioactivity data about them are not available [24].

6. CHALLENGES IN MOLECULAR DOCKING

There are many difficulties in using docking tools and the results of the study. It is reported that each program has its limitations and flaws [147]. Therefore, programs cannot provide the same output with the same reliability. Furthermore, the program may not perform well when the chemical structure processed exceeds the capacity of the developed software. Therefore, it is important to continuously validate and correct the developed software according to the new data. [127]. Considering all these, not surprisingly, the acceptance of the predictive tool results is still difficult. However, if the current problems of the tools are addressed properly, the value of the results and, therefore, the acceptance will increase. In addition to this, if the resolution of the protein structure available in the PDB or obtained from homology modeling is poor, the docking result might not be reliable. Thus, the selection of the structure of the protein to be used in docking should be done with great care [148].

6.1. Accuracy of Docking

Docking methods are widely used to identify possible ligands at the early stages of drug discovery and development. There are many programs used to elucidate the interaction of molecules with targets. Despite these programs, some molecules have not yielded promising results when they are tested *in vivo* [63]. Docking results may be interrogated due to diverse issues. The first one is related to the use of protein structure. Protein structures are generally available in complexes with ligands in the PDB [149]. Researchers delete the bound ligand to use the protein structure and do the docking of the molecule being investigated. On the other hand, this procedure may affect the docking output. The second important issue is the binding site environment. Drug candidate molecules must bind to targets within the cell to exhibit their activities. In some cases, even if the docking results exhibit high binding in the *in silico* environment, they may give a different result in the *in vivo* environment [63].

6.2. Properties of Ligand

It is impossible to predict the agonist or antagonist nature of a ligand by docking. Docking studies give information only about the binding mode and affinity of a molecule towards a receptor [20]. To check the agonist or antagonist properties of a molecule, experiments should be done in a laboratory after the docking process. Therefore, it is recommended not to overinterpret docking results regarding the nature of the ligand unless other validations like lab experiments, are performed [150].

Ligand preparation and conformation of ligands are also important in determining the docking results. In the ligand preparation, molecules are ionized prior to docking. However, the tautomeric state of the molecules is still a problem. There is no clear way of using the variable tautomeric states of the molecules to be docked [23].

6.3. Properties of Target

The quality of the structure of the target influences the reliability of the molecular docking. Molecular structures with the best geometrical parameters are chosen, but this doesn't guarantee that they are free of error. Thus, mechanisms of filtering that will help in ensuring the quality of the structures available in the databases, such as PDB are in need [151].

In the preparation of the target, solvents and ligands in the structure are usually removed. This leaves the binding pocket completely free. However, in the physiological state, the environment is different. This leads to a discrepancy between the two conditions [152]. In recent years, there are several attempts to use water molecules in the binding region. Nevertheless, there are still challenges in the way the water is put around the binding site [153].

There are docking programs that use rigid protein structures. In real conditions, the target structure can fluctuate depending on intrinsic and extrinsic factors, although it spends more time in the lower energy states. Thus, docking programs that keep the target rigid might give inaccurate results [153]. Using programs that allow the target structure to be flexible can be a solution here.

6.4. Search and Scoring Problems

Docking is difficult due to the various means of presenting two molecules in the 3D space together (three translational and three rotational degrees of freedom). The search algorithm implemented looks for all possible orientations between two molecules by systemically translating and rotating one molecule over the other [154]. Many solutions can be generated with a search algorithm. The solutions are ranked according to their scores [155]. There are diverse docking functions, and each program has its scoring system, so there is no universal scoring function [156]. In general, the correlation of docking scores with experimental binding affinities is still poor. Each docking algorithm uses a scoring function together with a search tool. Theoretically, the best matching algorithms and scoring functions should be merged to solve docking problems [157].

CONCLUSION

Molecular docking is a popular structure-based drug design method that predicts the interactions of small-molecule

ligands with the appropriate target. There are various powerful docking programs used for this purpose. Since no single program is suitable for every system, choosing the most appropriate one is recommended based on availability, need, and computer capacity.

Molecular docking has many applications at various stages of drug discovery. It has an established application, especially in virtual screening and drug repurposing. Besides the familiar diseases, there are several emerging diseases nowadays. Thus, there is an urgent need for the discovery of potent drugs against such diseases. Molecular docking is playing an important role in the discovery of such drugs.

The challenges and limitations of molecular docking are overcome by the involvement of other computational approaches. State-of-the-art computational methods like AI and ML are expected to contribute much more in the near future. Furthermore, with the increase in accessible biological and chemical data, its application field is widening. As a result, the use of molecular docking in the drug discovery process is increasing. As a reflection of this, the number of publications in this area has doubled almost every five years for the last two decades.

The latest developments in other computational approaches had a substantial impact on molecular docking. Therefore, there are advances in the quality of the generated ligand-target binding modes and affinities. This will increase the applicability of the resulting interactions. Thus, the role of molecular docking in making the drug discovery process rapid, economical, and more effective is expected to rise.

LIST OF ABBREVIATIONS

3CLpro	=	3C-Like Protease
3D	=	Three Dimensional
ACE2	=	Angiotensin-Converting Enzyme 2
AI	=	Artificial Intelligence
BEAR	=	Binding Estimation After Refinement
CADD	=	Computer-Aided Drug Design
CANDO	=	Computational Analysis of Novel Drug Opportunities
COVID-19	=	Corona Virus Disease 2019
CPU	=	Central Processing Unit
DRAR-CPI	=	Drug Repositioning and Adverse Reactions *via* Chemical Protein Interactome
E	=	Envelope
GPU	=	Graphics Processing Unit
HCV	=	Hepatitis C Virus
HGFR	=	Hepatocyte Growth Factor Receptor
HIV	=	Human Immunodeficiency Virus
M	=	Membrane
MD	=	Molecular Dynamics
ML	=	Machine Learning
MM	=	Molecular Mechanics
MM-PBSA	=	Molecular Mechanics–Poisson Boltzmann Surface Area
M^{pro}	=	Main Protease
N	=	Nucleocapsid
Nsp	=	Nonstructural Proteins
PDB	=	Protein Data Bank
PDGFR	=	Platelet-Derived Growth Factor Receptor
PDTD	=	Potential Drug Target Database
PLpro	=	Papain-Like Protease
QM	=	Quantum Mechanics
QSAR	=	Quantitative Structure-Activity Relationships
RdRp	=	RNA-dependent RNA Polymerase
RM	=	Random Forest
S	=	Spike
SARS-CoV-2	=	Severe Acute Respiratory Syndrome Coronavirus 2
SIDER	=	Side Effect Resource
SVM	=	Support Vector Machines
TMPRSS2	=	TransMembrane Protease Serine 2
TTD	=	Therapeutic Target Database

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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