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Molecular Dynamic Simulations and Molecular Docking as a Potential Way for Designed New Inhibitor Drug without Resistance

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Mycobacterium tuberculosis is the cause of tuberculosis in humans and is responsible for more than 2 million deaths per year. Despite the development of anti-tuberculosis drugs (Isoniazid, Rifampicin, Ethambutol, pyrazinamide, streptomycin, etc.) and the TB vaccine, this disease has claimed the lives of many people around the world. Drug resistance in this disease is increasing day by day. Conventional methods for discovering and developing drugs are usually time-consuming and expensive. Therefore, a better method is needed to identify, design, and manufacture TB drugs without drug resistance. Bioinformatics applications in obtaining new drugs at the structural level include studies of the mechanism of drug resistance, detection of drug interactions, and prediction of mutant protein structure. In the present study, computer-based approaches including molecular dynamics simulation and molecular docking as a novel and efficient method for the identification and investigation of new cases as well as the investigation of mutated proteins and compounds will be examined.

Key words: Molecular dynamic simulation; Molecular docking; Drug resistance; *Mycobacterium tuberculosis*; Drug design

INTRODUCTION

Tuberculosis (TB) is an infectious disease that is a major threat to public health (1-3). Responsible for 2 million deaths per year (one every 15 seconds) around the world, especially true in areas with high poverty, unfavorable living conditions, lack of adequate medical and primary care (4, 5). *Mycobacterium tuberculosis* (MTB) is the oldest known human pathogen affecting more than a quarter of the world's population. It is also more common in countries with poor resources (6, 7).

Despite the development of numerous anti-tuberculosis drugs (Isoniazid, Rifampicin, Ethambutol, streptomycin, pyrazinamide, etc.) and the TB vaccine, the disease has still claimed the lives of many people worldwide because effective treatments are either too long or expensive. According to the World Health Organization (WHO), resistance to at least two drugs, isoniazid (INH) and rifampin (RIF), causes multidrug-resistant tuberculosis (MDR-TB) (6, 8-11).

Antimicrobial resistance (AMR) is one of the most important human health concerns as well as a major challenge for global drug discovery programs, which is the inefficiency of antibiotic drugs against specific bacteria (12, 13). Antimicrobial resistance has been reported in three rising levels, multidrug resistance (MDR), extensive drug resistance (XDR) and pan-drug resistance (PDR) (12). AMR threatens millions of people around the world and has

rightly been declared a global threat by the World Economic Forum (13, 14).

The susceptibility of anti-tuberculosis treatments in MDR-TB has decreased due to various mutations in the target drug-gene. This has worsened despite the combination of TB-HIV and appearance of the emergence of multidrug-resistant (MDRTB), totally drug-resistant (TDR) and extensively drug-resistant (XDR) TB (15-17).

The methods commonly used to detect drugs are laborious, costly, and time-consuming, requiring at least 10 years and about $ 800 million to produce a new drug. These processes are often not successful due to the low hit rate, failure to fulfill the required absorption, distribution, metabolism, excretion, and toxicity (ADMET). Therefore, a better method is needed to produce TB drugs (4).

Computer-aided drug design through modeling and docking an alternative method useful for drug discovery and development in this field (18, 19). Also due to the availability of the complete MTB genome as well as the initial and third structures of the unique proteins required for the survival of this organism, this method means virtual screening using computational modeling due to cost reduction and time required to identify active drug cases it can be helpful (20).

As a result, bioinformatics approaches can be used to predict the structure of mutant proteins along with studies investigating the mechanism of drug resistance and revealing drug-target interactions at the structural level to obtain new drugs in the field.

In the present study, we intend to review computer-based approaches including molecular dynamics simulation and molecular docking to identify and investigate new cases for the design of drugs that affect mutant proteins and the metabolic pathways involved in these pathogens, together with some practical details in few examples.

MOLECULAR DYNAMICS (MD)

It can be stated that one of the most efficient and best methods of studying biological macromolecules is the Molecular dynamics (MD) simulation method (21-23). MD simulations of protein structure can be performed in an aqueous medium to provide predicted adaptations of proteins under physiological conditions (24-26).

They are also important for understanding the dynamic behavior of proteins based on different times (from fast internal movements to slow structural changes or even protein folding processes) (27). In fact, this system shows (predictions based on a general physics model governing interactions) how each atom in different molecular and protein systems moves over time (22). An important ability of this method is to record a wide range of simulations of important molecular biomass processes. These include ligand binding, deformation, and folding protein, representing the positions of all atoms in femtosecond resolution (28). On the other hand can examine the influence of explicit solvent molecules on protein structure and stability to obtain the average properties of the biomolecular system including density, conductivity, and dipole moment, as well as various thermodynamic parameters including interaction energy and entropy (29).

X-ray crystallography, cryo-EM (cryo-EM), nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), Förster or fluorescence resonance energy transfer (FRET) are structural biology techniques often used in combination with MD simulations (28).

The groundbreaking studies have shown a fundamental role in classical MD simulations in the study of biological systems. They used MD simulations to obtain various combinations of proteins and nucleic acids, including early attempts to spontaneously simulate complex phenomena such as protein folding.

Late in the 1950s, the first simulation was made of simple gases (30). The first simulations of a protein were made in the late 1970s (31), the factors that made these simulations possible were the achievements that received the Nobel Prize in Chemistry in 2013 (32, 33). The enormous increase in computing power permits simulation

of systems 104-106 atomic (34, 35) and simulation time from micro sec to nano sec, respectively (36).

However, MD simulations have become increasingly popular in recent years by the scientific community, especially experimental molecular biologists. With the recent advances in crystallography, tens of structures of different molecules have been identified (which were recognized by the Nobel Prize in 2003, 2012), whereas the crystallographic structure of membrane proteins has been difficult in the past. Cryo-EM (recognized by a 2017 Nobel Prize) was one of the solutions that accelerated the identification of such structures (37).

Over the past decade, due to the rapid development of faster architectures and better algorithms for performing high-level calculations on time (2 molecular dynamic base), we have seen an increase in the effectiveness of computational structure-based drug design (SBDD) in drug discovery. The introduction of new computer hardware, especially graphics processing units (GPUs), allowed powerful simulations to be run at an average cost locally (38, 39).

HOW MD SIMULATION WORKS

It can be said that the basic idea of this technique (MD simulation) is simple. Depending on the position of all atoms in a biomolecular system (Proteins that are surrounded by lipid bilayer or water can be an example), the force applied to each atom can be calculated by all other atoms (28).

Classical MD can be considered a physical method based on Newtonian physics to study the motion of atoms and molecules and the interaction between them. In this way, a force field is used to estimate the forces between the intersecting atoms and to calculate the total energy of the system (40). Then during MD simulation, Newton's laws of motion integration, sequential configurations, create a transformation system, providing paths that determine the positions and velocities of the particles over time (40). The general steps in an MD simulation are illustrated in Figure 1.

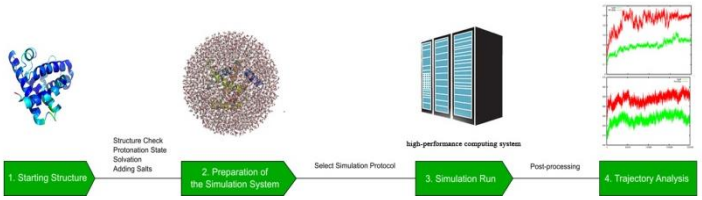

Figure 1. General steps in Molecular dynamics simulations that can be imagined.

The result of this path describes the configuration of the atomic level of the system at any point in time simulated as a 3D film. These simulations can be described as a powerful method for the following reasons: First, they record the location, mode, and rate of movement of each atom at any point in time, which is very difficult with any other laboratory method (28). Second, the simulation processes are very precise and can be controlled with high accuracy, including the primary composition of a protein to which the ligases are attached including a variety of post-translational and mutant changes, voltage, protonation, the temperature in a membrane that are present in the environment by other molecules, and so on. The impact of a wide range of molecular perturbations can be studied and compared by using the results of simulations performed under different conditions. The forces in the MD simulation are often calculated by molecular mechanical field modeling, which usually according to the experimental measurements and the results of quantum mechanical calculations. To ensure numerical and statistical accuracy of the numbers in an MD simulation, the time steps must be short, typically these are only a few cases of femtoseconds (10-15 seconds) (Figure 2). Most important biochemical processes (such as structural and functional changes of proteins) occur at nanoseconds, microseconds or longer. In any case, a typical simulation involves millions or billions of time steps. Alongside this, millions of interatomic interactions that are simultaneously evaluated in a single time step make the simulation processes highly computational. Recent improvements have been remarkable. Over the past few decades, longer and cheaper simulations have become available with advances in computational hardware,

software, and algorithms used for MD. Highly specialized hardware (41, 42) led to a significant increase in computing speed and made specific simulations possible in milliseconds (Figure 2).

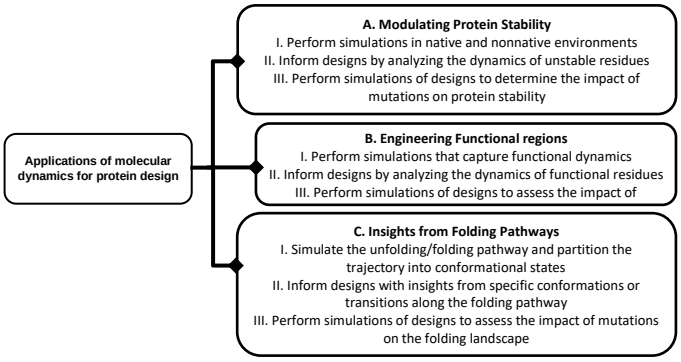

Figure 2. Applications of molecular dynamics for protein design. (A) Molecular dynamics simulations can be used to design stable protein variants, (B) engineer functional regions, or (C) provide insights from protein unfolding/folding pathways.

Perhaps most importantly, GPUs allowed simulations performed on one or two inexpensive PC chips to perform better than they had previously done on supercomputers (38). These GPUs have made simulations at significant biological intervals much more accessible to researchers than ever before. Simulations are now relatively straightforward, and computational resources for performing useful simulation values are increasingly available. Due to the large amounts of data on the different paths of atoms, obtaining accurate biological insights and interpreting simulation results can be challenging.

Evaluating the mobility and flexibility of different regions of a biomolecule is one of the most fundamental applications of these types of simulations. By simply simulating such a structure, one can determine how many different molecules move in equilibrium and what kinds of structural oscillations it undergoes. Other features of this method include simulating some of the functional behaviors of protein and ligand binding, which are often very important (dynamic behavior of water molecules and salt ions) (43-45).

They are also commonly used to refine as well as constructor refine structural models based on empirical structural biology data. (Often using MD protocols, the X-ray crystal structures fit the experimental data, preserving the model structure) (46).

In specific applications such as ligand and protein design, these types of simulations are mostly used as a relatively inexpensive filter to filter (binding or stability energy) large numbers of candidates in a small sample that can be tested (47-49).

On the other hand, more importantly, it can be pointed out that simulations lead to new laboratory work by presenting new hypotheses .The development of new drugs is an exciting new area where MD simulations can perform different experiments (50, 51).

They are also valuable in optimizing lead, whereby the performance or properties of lead in a ligand is improved or modified. These simulations can be used to predict the return of a ligand-binding pocket, to identify the key interactions of a ligand with the binding pocket site, as well as to identify possible ligand potentials (52, 53).

MD may also be useful for virtual screening, predicting where a selected primary ligand binds to the target. Virtual screening is usually traditionally performed using a single structure of a target protein by docking software (54).

Simulations may also help design drugs with the desired binding and dissociation kinetics of features that have recently been identified for efficacy and safety. For example, the effectiveness of ligands on specific targets is associated with residence time rather than binding affinity. In various studies, MD-based methods in ligand ranking have been studied according to their dissociation rate (55).

PRACTICAL TIPS ON USING MD SIMULATION

The most common computing hardware used is GPUs, which are a good option because of their fast simulation and average cost, but simulations are also performed on supercomputers using central processing units (cup) that can provide more speed. The most common types of forces used can include various versions of AMBER, CHARMM, and OPLS (56-59).

These forces each have their strengths and weaknesses, but they all rely on similar functional forms. For example, CHARMM36m and the CHARMM General Force Field (CGEFF) complement field have optimized many valid parameters for drug-like ligands, lipids, and proteins (58, 60, 61).

Also mentioned is the A99SB force field, which has been recently introduced for disrupted or disordered proteins (59) and OPLS3 is a force field that optimizes ligand parameters at best, although their specific nature generally precludes third-party evaluation (57). Common software used includes GROMACS, NAMD, AMBER, CHARMM, DESMOND, and OPENMM (62-65).

AMBER and CHARMM software should not be confused with the AMBER and CHARMM force fields. All modern software available for simulation supports different force fields. The packages mentioned are the same in terms of computation but they differ to support features and how to use different hardware (e.g., coarse-grained simulation support, temperature and pressure control schemes, and sampling methods).

One of the most important sources for MD simulations is the scientific research collaboration with structural bioinformatics (RCSB, WWW.rcsb.org), which makes available 3D macro logical biological structural data (66). The RCSB Protein Database (PDB) is a global repository for the processing and distribution of three-dimensional structure data of macromolecules, such as proteins and nucleic acids, and is an essential resource for biomolecular modeling (66).

It should be noted that the three-dimensional structure of various drug molecules is available in several large databases such as NIH (67), ZINC (68, 69), and Drug Bank (69).

It should be noted that the structures obtained from the experiments require some processing to prepare them for simulation (Including hydrogen atoms that are not generally soluble in crystalline structures) Also add some "solvents" such as lipids, water, ions, salt and determine the force parameters. Many common simulation software has been improved, and available to make system preparation easier (28, 70, 71). One of the challenges facing decision-makers in choosing the right type of simulation for each project as well as analyzing the results (including advanced sampling techniques for use where applicable). Analyzing MD simulation results for a variety of reasons can be challenging.

These simulations generate a lot of data. Typically a simulation can track the positions and velocity of 100,000 atoms over a billion-time step. Identifying the data and biological aspects of these data are very important and challenging. Several common "pre-packaged" analytics are readily available in the software, but most simulation projects benefit from writing custom analytics programs or scripts dramatically through multiple frameworks (56, 72-74).

Both MD simulation design and interpretation of the results have limitations: First, the force fields used in MD are inherently approximate, although they have improved greatly in recent years (75). Second, covalent bonds do not break and form during conventional MD simulations, meaning that the residual rotational states of the amino acids are titrable constant and must be carefully adjusted at the start of a simulation unless the approaches applying the pH simulation (76) constant is typically the case with a significant increase in computational cost for disulfide bands. Third, the availability of an accurate protein structure or a good matching model as a prerequisite can be considered an influential factor in the accuracy and efficiency of the simulation. Finally, it can be concluded that the design of simulation studies is strongly influenced by the availability of laboratory structures. As mentioned, MD simulations have become relatively simple in recent years, but MD simulations are still used indirectly to achieve high-impact conclusions. To perform quality and reliable work by MD, it is important to identify the research process by MD with appropriate empirical and computational studies and carefully tailor these simulations for them.

MOLECULAR DOCKING

Molecular docking is an essential part of the computer-aided drug design tool (77). It first appeared in the 1980s and early 1990s to predict the binding state of active compounds and the screening of large digital library complexes. This method was used to reduce costs and speed up the process of drug discovery, which is part of the "structure-based drug design" approach (78, 79).

Since the early 1980s, molecular docking has been the most common method of structure-based drug design (80). Programs based on various algorithms have been developed for molecular docking studies that have made docking increasingly important in pharmaceutical research.

Molecular docking is a method of analyzing the composition and orientation of molecules into the binding site of a macromolecular target. The search algorithm generates potential poses, which are ranked by scoring functions (81). There have been many good reviews of docking in the past (82-85), and many studies have been done to compare the relative performance of programs (86-89).

In this method (molecular docking), the behavior of small molecules at the junction of the target protein can be investigated and also used in interaction modeling between a small molecule and a protein at the atomic level and also identify the underlying biochemical processes (90, 91).

Over the past few decades several software applications have been developed, some of which are very popular, such as Autodock (92), Autoduckvina (93), DockThor (94, 95), GOLD (96, 97), Flexx (98) and Molegro Virtual Docker (99).

The docking process involves two basic steps: The composition of the ligand, its orientation, as well as its position is predicted in different sites (usually referred to as pose) (100, 101).

In most cases, the structures of macromolecules can be obtained from the Protein Data Bank (PDB) (102), which provides our access to the three-dimensional atomic coordinates obtained by experimental methods. It is also possible that the experimental 3d structure of the target is not available, but is not common. To overcome this problem, computational forecasting methods such as comparative and ab initio modeling can be used to obtain the three-dimensional structure of proteins (81).

Knowing the binding site before docking processes significantly increases docking efficiency (100). In many cases, the binding site is identified before ligand binding (100). One can also obtain site-related information by comparing the target protein with a family of proteins that function similarly or with crystallized proteins with other ligands (100).

If you do not know the connectivity sites, cavity detection programs or online servers for example: pass (103), SURFnet (104, 105), Pocket (106), GRID (107, 108) and MMC (109) can be used to identify putative active sites in proteins. Blind docking is a form of docking that is performed without any assumption about the junction (100). The site of the junction is usually specified to focus on docking calculations. However, when area information is missing, there are two common approaches: either the most probable algorithmically predicted sites or "docking blind" simulations (110).

Several existing software can be used to identify binding sites. For example, moldock (99) uses an integrated cavity detection algorithm to identify potential binding sites. DoGsiteScore is an algorithm that determines possible pockets and their druggability scores, which describes the potential of a binding site to interact with a small Drug-like molecule (111).

During docking calculations, one strategy is to use a network that includes predefined potential energies for interaction at the target junction (83). This method speeds up the execution of docking and essentially involves discretization of the junction (112).

Ligand structure is also required and small molecule databases such as ZINC (69) and PubChem (67) can be used. However, better evaluation of rotations, free tarsions,

protonation states, and charge assignments is crucial for successful docking.

Two things are important in system docking: Ranking scoring functions and search algorithms. The analysis of the search algorithm and generates ligand pose at the junction is a goal, With regard to roto-translational and internal degrees of freedom of the ligand (101).

Search strategies are often classified as systematic, random, or definitive (83).

The systematic search algorithm gradually evaluates the release rate of each ligand (83, 113).

For example, component-based methods with systematic algorithms used in Flexx and eHits (114) can be noted. Various algorithms have also been developed to use pharmacophore information related to proteins and ligands. These algorithms attempt to coordinate the distance between the pharmacophoric points of the ligand and the protein from the pharmacopoeial match (115).

For example, FIEXX-PHARM software is an extensive version of FLEXX and uses pharmaceutical features as a limitation in docking calculation (111).

Random search algorithms make changes in the degree of ligand release.

Some software, such as AutoDuck, GOLD, DockThor, and MolDock, use random algorithms for search methods (79).

Although the challenges and limitations of the docking method have been identified in the last two decades (87), this research topic is still very active.

COMBINED DOCKING AND MD SIMULATION

For more reliable results of protein-ligand complexes, a combination of cheap and fast docking methods with accurate but expensive MD techniques can be used. The strength of this compound lies in its complementary strengths and weaknesses.

On the one hand, docking techniques are used to discover the vast conformational space of ligands over a short period and allow for the careful examination of large libraries of compounds such as drugs at a reasonable cost. The major disadvantages are the lack or poor flexibility of the protein that does not allow for the regulation of its composition on ligand binding and the absence of a unique and widely applicable function necessary to establish a valid ranking of the final complexes. On the other hand, MD simulations can flexibly treat both ligands and proteins, allowing for the suitability of the receptor-binding site around the newly introduced ligand. Therefore, a combination of the two protocols in which docking is used for rapid screening of large libraries and MD simulations are used to detect protein receptor structures, optimize the structure of end complexes, and calculate precision energy, an approach It is reasonable to improve the drug design process.

CASE STUDY

Here we are going to show you how to do structural analysis by reviewing some studies in this field. Isa et al., 2018 conducted a study to investigate the 3-dehydroquinate synthase (DHQS) pathway using in Silico docking and molecular dynamic simulation. This pathway is important because it is present in bacteria, algae, fungi, and plants but does not occur in mammals. The shikimate pathway is an important and integral pathway for the metabolism of MTB (naphthoquinones, menaquinones, and mycobactin biosynthesis). In this study, novel inhibitors of 3-dehydroquinate synthase (DHQS) were identified, an enzyme that catalyzes the second stage of the sheik pathway in MTB. A total of 18 compounds with the best binding energies were selected from 12,168 compounds from two public databases through virtual screening and molecular docking analysis using PyRx 8.0 and Autoduck 4.2. These 18 compounds were analyzed and screened for absorption, distribution, metabolism, excretion, and toxicity (ADMET) and found 9 compounds that satisfied all ADMET criteria. Among the various compounds, three compounds with the best binding energy were selected to molecular dynamics simulation. Finally, the two compounds ZINC633887 and

PubChem73393 formed stable complexes with DHQS and the structure of the two ligands remained largely unchanged during the simulations at the ligand-binding site. The two compounds identified by these methods (docking and MD simulation) are potential candidates for the treatment of tuberculosis that must be approved in -vivo and in-vitro (4).

In another study, Kumar et al., in 2017 investigated computer simulations of susceptible L, D Transpeptidase by Carbapenems and Cephalosporins in *Mycobacterium abscessus*. The importance of L, D Transpeptidase is because most of the linkages in the cell wall peptidoglycan of M. abscessus are synthesized by non-classical transpeptidases. In this study, the interaction of β-lactams with two L, D transpeptidases in M. abscessus, LdtMab1 and LdtMab2 was investigated and found that both Carbapenems and Cephalosporins, not Penicillins, inhibited these enzymes (116).

Halder et al. (2019) used in Silico absorption and multiple docking analysis to investigate the ADMET of anti-leprosy and Dapsone compounds against the synthesis of Dihydropteroate synthase from *mycobacterium leprea* (117). Because Dapsone is an expensive antibacterial drug that has many side effects, a natural and cheaper alternative is needed. The three-dimensional protein structure of the dihydropteroata synthase was modeled from M. leprae. All analytical docking analyzes were performed using AutoDock Vina, AutoDock 4.2.6, and SwissDock. The result showed that neobavaisoflavone tends to bind better than Dapsone and forms a stable protein-ligand complex (117).

Another use of the molecular docking system by Tuhin Ali et al. (2018) on the anti-TB potential of propolis selective elements can be mentioned (118). propolis, a substance naturally produced by bees after collecting herbal resins, is used in folk medicine for its beneficial anti-tuberculosis activities. In this study, investigated the interaction between selected propolis compounds and four "druggable" proteins that are critical for the function of TB physiology, namely MtPank, MtDprE1, MtPknB and MtKasA using molecular docking (118). As a result, both the combination of MtDprE1 and MtKasA showed superior docking scores than control inhibitors and provided interesting potential scaffolds for in vitro biological evaluation and anti-TB drug design (118).

CONCLUSION

As mentioned earlier, with the development of anti-TB drugs (Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, Streptomycin, etc.) and the TB vaccine, the disease continues to threaten the lives of many people around the world. Drug resistance in this type of disease is increasing day by day. Appropriate, fast, and efficient methods are needed to identify and design new drugs without drug resistance. Bioinformatics approaches can be used to predict the structure of mutant proteins, along with studies of the mechanism of drug resistance and the identification of drug-mediated interactions with a structural target, to obtain novel drugs in the field. These approaches include molecular docking techniques and molecular dynamics simulations. The rigorous use of MD simulations in conjunction with complementary empirical methods now shows an area of great opportunity in the various sciences. Effective use of simulations in molecular biology and drug discovery requires careful thinking about existing experimental and computational data, and thus benefits from both extensive expertise and interdisciplinary collaboration. It is also important to note that each of these techniques has its drawbacks and weaknesses but new approaches that use a combination of the two will improve prediction performance and allow for better utilization of information in the future. It is hoped that using these new approaches will be able to design and manufacture effective drugs without resistance.

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