Molecular Dynamics for Antimicrobial Peptide Discovery

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ABSTRACT Although antimicrobial resistance is an increasingly significant public health concern, there have only been two new classes of antibiotics approved for human use since the 1960s. Understanding the mechanisms of action of antibiotics is critical for novel antibiotic discovery, but novel approaches are needed that do not exclusively rely on experiments. Molecular dynamics simulation is a computational tool that uses simple models of the atoms in a system to discover nanoscale insights into the dynamic relationship between mechanism and biological function. Such insights can lay the framework for elucidating the mechanism of action and optimizing antibiotic templates. Antimicrobial peptides represent a promising solution to escalating antimicrobial resistance, given their lesser tendency to induce resistance than that of small-molecule antibiotics. Simulations of these agents have already revealed how they interact with bacterial membranes and the underlying physiochemical features directing their structure and function. In this minireview, we discuss how traditional molecular dynamics simulation works and its role and potential for the development of new antibiotic candidates with an emphasis on antimicrobial peptides.

KEYWORDS antimicrobial peptides, molecular dynamics, computational biology

Antimicrobial-resistant bacteria pose an increasingly significant threat to global public health. While resistance is increasing, only two truly new classes of antibiotics have been approved for clinical use since the 1960s (1). The World Health Organization estimates that by 2050, 10 million deaths per year could be attributable to antimicrobial-resistant bacterial strains (2). Even today, secondary bacterial infections pose complications for other diseases, with bacterial coinfection playing a critical role in the current severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) pandemic (3–5). Therefore, new ways to rapidly develop and test novel antibiotics are needed to the global health crisis of antibiotic resistance.

Antimicrobial peptides (AMPs) represent a promising alternative to conventional antibiotics (6). Since their discovery, AMPs have been found in a diverse range of life forms, from bacteria to humans, as a natural form of defense against disease (7, 8). There are now over 20,000 synthetic and natural AMPs contained in the DRAMP database (9), and this number is rapidly increasing. The advantage of these peptides over traditional antibiotics is that they act through multiple mechanisms of action (10), sometimes with no specific bacterial targets, thus decreasing susceptibility to resistance development. AMPs typically act on the bacterial membrane, making it difficult for the pathogen to adapt and develop resistance mechanisms (7). In addition to their

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antibacterial effect, AMPs variably display antiviral (11), antifungal (12), anticancer (13, 14), and immunomodulatory (8, 15) activities. The wide variety of targets and functional versatility of AMPs have made them successful scaffolds for many therapeutic applications over the past 4 decades, yet relatively few have reached the clinic (16). There are several drawbacks to using AMPs for therapeutic applications and last resort treatment, including their propensity for toxicity toward human cells, lack of systemic efficacy, and poor absorption properties. Efforts are now under way to create synthetic AMP derivatives with reduced eukaryotic cell toxicity while maintaining their efficacy against microorganisms, including antibiotic-resistant bacteria (10, 15, 17).

AMPs interact with bacteria and cause cell death in a multitude of ways (18). Understanding these mechanisms is critical for future AMP-based antibiotic development. However, it is challenging to study the mechanisms of action of these agents because of the transient nature by which they typically function, which can limit the effectiveness of experimental techniques (19). Recent advances in computational power have vastly increased the applicability of computers in aiding the design of novel potential medicines (20–23). Molecular dynamics (MD) simulations constitute a primary avenue for computational exploration of AMP mechanisms of action. Simulations have reached incredible speeds and involve increasingly large systems. Systems reaching hundreds of thousands of atoms are now regularly simulated on millisecond-long time scales (24–26). Remarkably, even systems up to 100 million atoms in size have been simulated for 500 ns (27). MD allows for the sampling and observation of conformational space of a nanoscopic system. The careful analysis of this conformational space reveals great insights into the dynamic characteristics of the complex system (28). Here, we review the capabilities of MD to lend itself to novel antibiotic discovery, particularly by illuminating AMP mechanisms of action.

A BRIEF INTRODUCTION TO MOLECULAR DYNAMICS

MD has found broad utility in the study of proteins, from characterizing their simple movements in a solution to simulating folding. More broadly, applying MD to drug discovery is increasingly common, facilitated by enhanced computational power, more efficient and accurate algorithms, greater ease of use, and the creation of large-scale macromolecule structure data banks (29). MD has been used to study a large number of new drugs and to gain substantial insights into the nanoscopic mechanisms of proteins, nucleic acids, and many other biological systems that interact with drugs. This computational tool enables rapid and inexpensive molecular screening, reducing resources spent on large physical experiments. For example, MD has been coupled with ligand docking methods to rapidly screen for new SARS-COV-2 protease inhibitors among drugs already approved by the U.S. Food and Drug Administration (FDA) (30, 31). However, it should be noted that these screenings are based on very specific intermolecular interactions. MD studies of AMPs are usually more complex because of the nonspecific interactions that occur when these agents are in contact with the membrane, as well as the various properties (e.g., fluidity and lipid composition) of the membranes with which they interact.

There is a plethora of MD software available (e.g., NAMD [26], GROMACS [32], or AMBER [33]), most of which leverage new developments in graphics processing unit (GPU)-accelerated computing. Large-scale simulations are now consistently reaching the microsecond or millisecond range. Classical MD uses a simple potential energy function to describe the motion of particles. Bonds between particles are modeled as springs, with a different function for bonds, angles, proper dihedrals, and improper dihedrals. Potential energy contributions of nonbonded Van der Waals interactions using Lennard-Jones potential and electrostatic interactions using Coulomb's law are also contemplated. Force constants for every type of atom and bond are described by a molecular force field that is commonly informed through experimental techniques or quantum calculations (34). The choice of force field is highly important for simulating a given system of interest. Most MD software available has specific versions of these force fields that work

optimally with the given software. Another consideration is that some molecules are better described in certain force fields, which may somewhat complicate the choice of software. This consideration is particularly important, since properties of a given biological environment, such as ion concentration, can drastically alter the effectiveness of AMPs (35). By combining bonded and nonbonded forces with all-atom simulations, researchers can discover small interactions between molecules that would otherwise be extremely difficult to determine using conventional experimentation. In order to apply MD to antibiotic development, we need to develop well described force fields for new molecules. In addition, these force fields do not include the behavior of electrons and cannot involve systems requiring covalent reactions. Hybrid quantum mechanical/molecular mechanics (QM/MM) approaches can be leveraged when the behavior of electrons is required for proper understanding of the system, but this approach is not covered in this review (36).

The typical MD simulation is run with periodic boundary conditions, where copies of the system are effectively simulated infinitely in all directions to reduce artifacts generated by simulating small systems with a perfectly defined geometry. To sum long-range electrostatic effects over these periodic boundary conditions, the particle mesh Ewald (PME) method is typically used. This method is powerful for reducing computational load and increasing accuracy, yet it requires certain restraints which can limit the types of systems that can be simulated. PME requires that the entire system be at neutral charge so that no artifacts are generated. For example, a peptide with a net charge of +4 will require ions with a collective charge of -4 to counterbalance the positive charge. In addition to the periodic boundary conditions, there are several ensembles that are typically used to constrain the volume, energy, pressure, and temperature to improve the accuracy of a simulation. The microcanonical NVE ensemble controls molar quantities (N), volume (V), and energy (E); the canonical NVT controls molar quantities (N), volume (V), and temperature (T); the isothermal-isobaric NPT controls molar quantities (N), pressure (P), and temperature (T). The most common ensemble, NPT, requires the use of a thermostat and a barostat, the choice of which can vary depending on the software and what most accurately reproduces the physiochemical properties of the desired molecular system. Since the activity of AMPs often varies between different complex biological systems, choosing this system at the molecular level is particularly relevant for understanding the mechanisms of action of AMPs. In particular, MD simulations of membranes are important for AMPs, as many of them interact with different lipid compositions in a number of ways. For instance, a common use of MD for studying AMPs is to determine how they react differently toward eukaryotic and bacterial membranes (37, 38).

While MD has proven to be a valuable tool to understand nanoscale interactions, certain challenges and limitations still exist. While computation has advanced significantly over recent years, long simulations on large systems still require a moderately expensive computer or time allocations on a supercomputer such as ANTON2 (39). In addition, even a long simulation may not capture the full conformational space of a given system. MD simulations do not represent all possible trajectories that a system can undergo. These trajectories show only snapshots of conformational space in which a dynamic system evolves. Thus, a system that requires long timescale conformational changes or large energy barriers may not be sufficiently sampled in MD simulations. These issues may be resolved with enhanced sampling techniques like steered molecular dynamics (SMD), where a force applied to a structure can enable a system to sample a larger variety of conformational space with less computational effort. Another MD method that has been used to simulate very large systems on microsecond to millisecond time scales is coarse-grained MD (40). In coarse-grained MD, groups of atoms are collected in "beads" and simulated as a single entity, thereby reducing the degrees of freedom of those atoms. This type of simulation is used mostly to study large changes in a system where small fluctuations in these groups of atoms are less important for understanding the experiment. Coarse-grained MD has been used successfully to study systems as large as an entire organelle (41). While these methods are powerful and can

FIG 1 Historical trends of using molecular dynamics to study antimicrobial peptides. The graph was generated by searching PubMed (<https://pubmed.ncbi.nlm.nih.gov>) for journal articles mentioning MD, AMP, and both AMP and MD in their titles and/or abstracts. The number of publications in a given year is not cumulative.

be effective, they must be used with caution and with careful consideration for what is being tested in a simulation.

The use of MD to study antibiotics has been increasing in recent years. Figure 1 shows the recent historical trends of using MD to study AMPs in comparison to all AMP or MD studies. This uptrend is likely due to the importance of physiochemical and dynamical structure for tuning the antimicrobial properties of AMPs. In addition, one area that is well studied in MD is lipid dynamics, which is also showing an important effect on the antimicrobial properties of AMPs (42). The precise way in which AMPs interact with lipids can vastly change their mechanism of action and effectiveness. By studying these interactions with MD, certain motifs that give rise to specificity against a particular target membrane can be identified, and this information can be used to inform the design of optimized engineered AMPs. Motifs that cause both antimicrobial activity and toxicity for human cells can be tuned *in silico* and studied using MD prior to empirical studies. Further experimental studies can then leverage what has been discovered with MD to reduce peptide toxicity for human cells and increase their activity against bacterial cells (15, 22, 43).

MOLECULAR DYNAMICS AS A TOOL FOR ANTIBIOTIC DISCOVERY

Most applications of MD to traditional drug discovery have involved studying small molecules after a docking screen of large libraries of molecules to a target protein (44, 45). Since the goal of a docking screen is to filter large libraries for potential targets, it makes certain assumptions about the binding relationship between protein and ligands to reduce computational load. One assumption is that the ligand fits into a protein like a “lock and key,” ignoring the flexibility of the protein and the ligand.

Furthermore, docking algorithms can have false-positive hits because they often disregard the full dynamics of a system. Since MD is one of the best computational methods for studying protein-ligand interactions, it is often used following a docking run to determine a more accurate binding energy (45).

Fully understanding the mechanism of action of a given molecule is a critical step in the drug discovery process. Kim et al. used MD to design a new class of synthetic retinoid antibiotics that proved to be effective against methicillin-resistant *Staphylococcus aureus* (MRSA) persister cells (46). In this study, a high-throughput screen of synthetic molecules was tested in a *Caenorhabditis elegans* infection model to identify two synthetic retinoids capable of decreasing MRSA-induced fatality. MD was then used to study how these molecules interact with the bacterial membrane, providing insight into why other analogues did not have antimicrobial properties. These results motivated the synthesis of several similar molecules, precipitating the discovery of additional potent antibiotics.

The free energy of binding between a ligand and a protein can be determined through the use of multiple MD methods, but free energy can be measured for many other changes in a system (47). For example, the changes in free energy from binding to a lipid membrane or the dimerization of two peptides in a membrane have been computed (38, 48). There are two overarching methods to calculate free binding energy. The first is called a physical pathway, because it can occur in a real system; the second is called an alchemical pathway, because the system must be modified in an artificial way to determine the free binding energy. Free energy may be calculated by computing the work that is required to remove a ligand from a binding site or environment and then to put it into solution. This method could be physical or alchemical, depending on the system being simulated. One alchemical method artificially changes a ligand to an analogue and measures the relative free binding energy. There are even more methods and techniques to analyze the free binding energies, which have been adequately reviewed (47).

MOLECULAR DYNAMICS FOR THE STUDY OF ANTIMICROBIAL PEPTIDES

There are several families of AMPs, defined primarily by their secondary structure and sequence: α -helical structures, random coils, β -turns, and peptides with mixed α and β secondary structures (49–51). However, these families do not exhaustively describe all peptide types, and AMP structure can vary depending on the environment to which a peptide conforms. Linear α -helical peptides are the most well-studied family of AMPs, comprising approximately 90% of all such peptides. Their diverse, short, amphipathic sequences are easier to synthesize and widely observed in natural host defense systems, e.g., in the venoms of insects and arachnids (10, 52–54). AMPs display many mechanisms of action (10, 49), which can be broadly classified into direct target and membrane-targeted mechanisms. One direct target mechanism involves AMPs crossing the lipid bilayer and disrupting intracellular functions (55), eventually resulting in cell death. Another way AMPs can fight infections is by modulating the immune response of host cells (56, 57). Membrane-targeting mechanisms usually involve destabilization of the membrane by the formation of pores (58), a “carpet” mechanism (58), nonlytic membrane depolarization (59), and membrane thickening and thinning (60). The barrel stave pore model involves the embedding of the AMP directly into the membrane without significant rearrangement of the lipids themselves, while in the toroidal pore model, the lipid head groups are pulled in with the AMP and cause significant membrane curvature. Another model of pore formation involves the targeting of peptidoglycan lipids present on the Gram-negative outer membrane. A visualization of certain mechanism-of-action models is shown in Fig. 2. It is also important to note that AMPs may act via additional mechanisms (49).

Since AMPs commonly act on the membrane, many MD simulations involve the simulation and dynamics of a lipid bilayer in conjunction with peptides. Investigation of comparative effects on human and bacterial cell membranes is important for designing optimized, target-specific AMPs. Many studies have compared the effects of

FIG 2 Mechanisms of action by which an AMP induces bacterial cell death. MD can be used to study a wide variety of AMP mechanisms of action. (a) AMPs can act on intracellular targets like nucleic acids or proteins, disrupting their functionality. (b) In the barrel stave pore model, AMPs are inserted into the membrane. (c) In the toroidal pore model, AMPs drag the lipid head groups into the membrane with them, creating a high membrane curvature. (d) In the detergent-like or “carpet” mechanism, AMPs directly destroy the bacterial membrane. (e) AMPs can act to thin the membrane, causing significant disruption to bacterial homeostasis. (f) AMPs can form disordered toroidal pores, permeating the membrane. (g) AMPs can also modulate the immune response by attracting immune defense agents or triggering the immune response, indirectly resolving infections. Figure 2 was created using BioRender software.

AMPs on diverse lipid bilayers. For example, it has been shown that varying the width of the bilayer directly impacts the dimerization and ultimately the antimicrobial effect of gramicidin embedded within the bacterial membrane (38). This study found that with increasing bilayer width, the energetic barrier of dimerization was increased. The authors corroborated this observation with fluorescence quenching experimentation showing reduced dimerization and therefore permeabilization with increasing bilayer width. Other studies have shown that the binding of peptides has different effects on the lipid dynamics of a membrane depending on its lipid composition.

Chen and Mark used MD to test the effect of membrane curvature on various mechanisms of AMP activity (61). This experiment tested aurein 1.2, citropin 1.1, maculatin 1.1, and caerin 1.1, each of which had been studied previously with physical experimentation such as circular dichroism or nuclear magnetic resonance (NMR) to determine their secondary structure in various environments. MD results were validated by verifying experimental data on the three-dimensional structure that the peptides exhibit in water and 2,2,2-trifluoroethanol (TFE). From there, multiple MD simulations with curved and flat 1-palmitoyl-2-oleoyl-glycero-2-phosphocholine (POPC) membranes were performed. Though limited by the computational power of the early 2010s, this study found that peptides are more likely to bind to curved membranes than flat ones, that aurein 1.2 and citropin 1.1 most likely act by a carpet mechanism, and that maculatin 1.1 and caerin 1.1 form pores. The experimental information revealed in this study allowed for the identification of specific motifs that cause the different mechanisms of these AMPs, which can be further adjusted and reprogrammed for optimized activity and eventual clinical applications.

Polymyxin B is a well-studied AMP that has been in clinical use since the early 1960s for the treatment of serious Gram-negative infections (62). This antibiotic has been studied since its discovery in 1947 (62, 63) and is one of the few AMPs that has seen widespread use. Long-term use of polymyxin B has prompted researchers to use it as a scaffold to design more optimal AMPs with fewer negative side effects. The structure of the polymyxin family of AMPs is particularly interesting because it deviates significantly from classical AMPs, containing multiple noncanonical residues such as D-amino acids, a ring structure, and a lipid tail (64). The structural deviations of the polymyxin family

are thought to aid in their ability to permeabilize the bacterial membrane. Noncanonical amino acids present within polymyxin B can be instructive for the design of synthetic AMPs, through the introduction of residues with chemical properties beyond those limited by the 20 canonical amino acids (10). For example, D-amino acids in polymyxin can prevent proteolytic degradation and could be added to synthetic AMPs for the same effect. Polymyxin B has also been shown to bind well to membranes containing lipid A, a component of lipopolysaccharides (LPS) present on the membrane of Gram-negative bacteria (65, 66). Unfortunately, although AMPs are less prone to antimicrobial resistance than traditional antibiotics, polymyxin B resistance has emerged in certain clinically relevant bacterial strains via complex restructuring of the LPS (67). Understanding the complex interactions that cause polymyxin B to bind to these bacterial membranes and why LPS restructuring disrupts these interactions is a task well suited for MD. As expected for most AMPs, polymyxin B has different mechanisms on both the inner and outer bacterial membranes. Berglund et al. has demonstrated through MD that polymyxin B aggregates on the outer membrane by interacting with the negatively charged LPS, while it interacts with the inner membrane primarily through hydrophobic interactions (68). A more recent study has confirmed the role of LPS and total membrane charge in determining the effectiveness of polymyxin B in binding to the membrane (66). In addition, studies also found that lipid packing in the membrane may play an important role in bacterial susceptibility to polymyxin B (64, 66, 69). Another important question for the design of AMPs is how to specifically target bacterial cells while reducing the number of deleterious interactions with human cells. Khondker et al. has shown using MD and experimental techniques that the presence of cholesterol in membranes reduces membrane permeabilization by polymyxin B through stabilization of the membrane and reduction of AMP packing in the bilayer (70). Continued study of the effect of membrane variation on the antimicrobial potential of this important AMP is critical for its subsequent development.

Certain AMPs have been shown to have increased antimicrobial properties when in the presence of metal ions (71). The innate immune system is known to use zinc (II) ions in multiple ways, including for immune modulation through interactions with natural colocalized AMPs. These types of AMPs that have synergistic effects with metal ions are collectively known as metallo-AMPs. Clavanin A, isolated from tunicates, is an example of an AMP that has potent intrinsic antimicrobial properties but that has displayed a 16-fold increase in activity in the presence of zinc(II) ions (72). An MD study of clavanin A found that zinc(II) binding to a common HXXXH motif (where H stands for histidine and X stands for any amino acid) induced α -helical conformations and even aided in chaperoning the peptide to the negatively charged lipid surface of a model bacterial membrane (72, 73). The same study observed that nearly all AMPs identified with the HXXXH motif had a similar synergistic effect with zinc(II). By carefully designing peptides with this motif, similar synergistic effects could be engineered into synthetic peptides.

CONCLUSIONS

The upcoming challenges of a post-antibiotic world are daunting, necessitating the accelerated discovery of novel antibiotic candidates. Computational techniques are especially poised to uncover underexploited modes of action for infectious disease therapeutics. MD is finding increasing utility in computational drug discovery and has been used to gain insight into diverse antibiotic mechanisms of action. Generalized drug development has benefited from MD simulations as an inexpensive method to determine important thermodynamic measurements, and this benefit is expected to be leveraged increasingly for antibiotic development. The use of MD has proven to be a powerful technique for illuminating the mechanisms of action underlying AMP activity. Studying molecules bound to membranes with MD is commonplace, and force fields for amino acids are well developed, leading to a

natural fit between MD and AMP discovery. Further study of AMPs with MD will greatly aid the study of structural motifs that can be used to design peptides with desirable therapeutic properties. Application of MD to peptide design is expected to progress as computational power expands, facilitated by more efficient algorithms, more advanced GPUs, and larger computational infrastructure.

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