Computational Methods and Tools in Antimicrobial Peptide Research

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# Abstract

The evolution of antibiotic-resistant bacteria is an ongoing and troubling development that has increased the number of diseases and infections that risk going untreated. There is an urgent need to develop alternative strategies and treatments to address this issue. One class of molecules that is attracting significant interest is that of AntiMicrobial Peptides (AMPs). Their design and development has been aided considerably by the applications of molecular models, and we review these here. These methods include the use of tools to explore the relationships between their structures, dynamics and functions, and the increasing application of machine learning and molecular dynamics simulations. The review compiles resources such as AMP databases, AMP-related web servers, and commonly used techniques, together aimed at aiding researchers in the area towards complementing experimental studies with computational approaches.

**Keywords:** Antimicrobial peptides, peptides, peptide engineering, antibiotic resistance, molecular dynamics, computational chemistry, membranes, aggregation, machine learning, artificial intelligence

# Introduction

One of the most important classes of drugs are antibiotics, which have found consistent use in medicine since their discovery in the first half of the twentieth century1. Antibiotics have provided an effective and powerful treatment for many bacterial diseases and remain amongst the most frequently used and prescribed drugs worldwide2. Unfortunately, due to their unregulated overuse, noncompliance, and high demand within the agricultural/animal industry, the emergence of antibiotic resistant bacteria has become problematic, such as methicillin-resistant Staphylococcus aureus (MRSA)3. This has resulted in loss of efficacy of the commonly prescribed antibiotics against previously treatable illnesses. To obviate this problem, new paradigms and approaches are needed, both to treat currently resistant infections and to reduce the risk of future resistance.

One approach has been to harness lessons learnt from a class of molecules that are naturally abundant. These are the AntiMicrobial Peptides (AMP) that exploit a mechanism that is different from those employed by more traditional antibiotics4. AMPs are used by the immune systems of many organisms to defend against bacterial infections by targeting and disrupting bacterial cell membranes. This contrasts with the traditional antibiotics, which themselves are derivatives of molecules produced by organisms as part of their defence, but generally target intracellular pathways rather than the membrane1, although membrane-targeting antibiotics have also been reported5. As the membrane is an essential component of the bacterial cell, the general hypothesis is that bacteria cannot easily remodel it, which significantly lessens the risk of developing resistance to AMPs. Indeed, since their discovery in the mid-twentieth century6–8, they have become an established class of antibiotics9. The earliest AMP, Gramicidin S, was first isolated from Russian soil by Gause and Brazhnikova in 1942 and was effectively used as a topical antibiotic to treat infected wounds by the Soviet Union in World War II10,11. Despite being one of the first antibiotics used in modern medicine, there is very little resistance reported for Gramicidin S, which has led to interest in understanding its mechanisms. It is now acknowledged that Gramicidin S affects bacterial cell membranes in a multifaceted manner, hence the low incidence of resistance11.

There are a variety of theories reflecting on the mechanisms underscoring the observed reduced susceptibility of AMPs to antibiotic resistance, which makes their role in modern antibiotic research vital. For example, AMPs have been shown to kill bacteria through multiple, diverse mechanisms, which is important because a diverse approach reduces the likelihood that site-specific bacterial mutations will result in resistance12. While traditional small-molecule antibiotics have specific intracellular targets, AMPs attack bacteria in multiple ways, both intracellularly and extracellullarly. They use a variety of membrane disruption mechanisms (these are discussed in more detail later and are the main focus of this review), as well interact with internal targets such as bacterial enzymes and nucleic acids, and stimulate the host immune response12. The targeting of bacterial membranes by AMPs is beneficial not only for diversifying strategies of attack, but also because the membrane is an essential component of the bacterial cell, making it difficult for the bacteria to effectively remodel it13. Furthermore, AMPs attack bacteria quickly and strongly13, and it has been reported that some AMPs repress the bacterial SOS-response14; both of these effects reduce the ability of bacteria to develop resistance pathways. Finally, it is interesting to note that many host-defense AMPs have co-evolved alongside bacteria, meaning that a multitude of AMPs are encoded in nature that were “designed” to evade bacterial resistance13,15. Understanding how the properties of AMPs depend on amino-acid sequence could give modern approaches the ability to respond to the emergence of new antibiotic resistant bacterial strains through the targeted tweaking of AMP sequences.

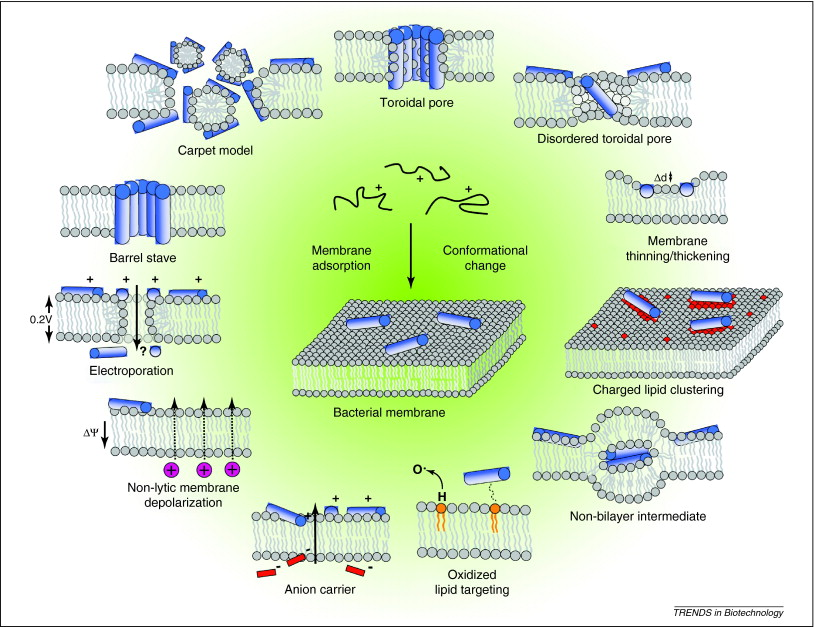


Figure 1: A graphical summary of AMP modes of action. Image from Nguyen et al.16. Used with permission.

AMPs are short peptides (10 to 50 residues), largely characterized by amphipathicity (possessing both hydrophilic and hydrophobic regions), and cationicity4,17. The combination of these properties allows them to selectively interact and disrupt the anionic bacterial cytoplasmic membranes through a variety of possible mechanisms, leaving the zwitterionic host membranes unaffected. Some of the most common modes of actions include membrane defragmentation (e.g., carpet model) and classical pore formation (e.g., toroidal pore and barrel stave models) (Figure 1). In addition, some peptides such as colistin and polymixin can also specifically target the outer membrane of Gram negative bacteria resulting in permeabilization18,19; a comprehensive review can be found in Nguyen et al[11](https://paperpile.com/c/lZKXdS/9w1iI). The exact mechanisms underlying antimicrobial activity, however, remain only partially understood, and unsurprisingly there are exceptions to the rules of thumb outlined above: for example, dermcidin is anionic20, and Gloverin is 130 residues long21. Additionally, the mechanism of action of an AMP appears to be determined by its secondary and tertiary structures, which can be ɑ-helical, β-stranded, mixed helical and stranded, or involve extended structures22 (Figure 2).

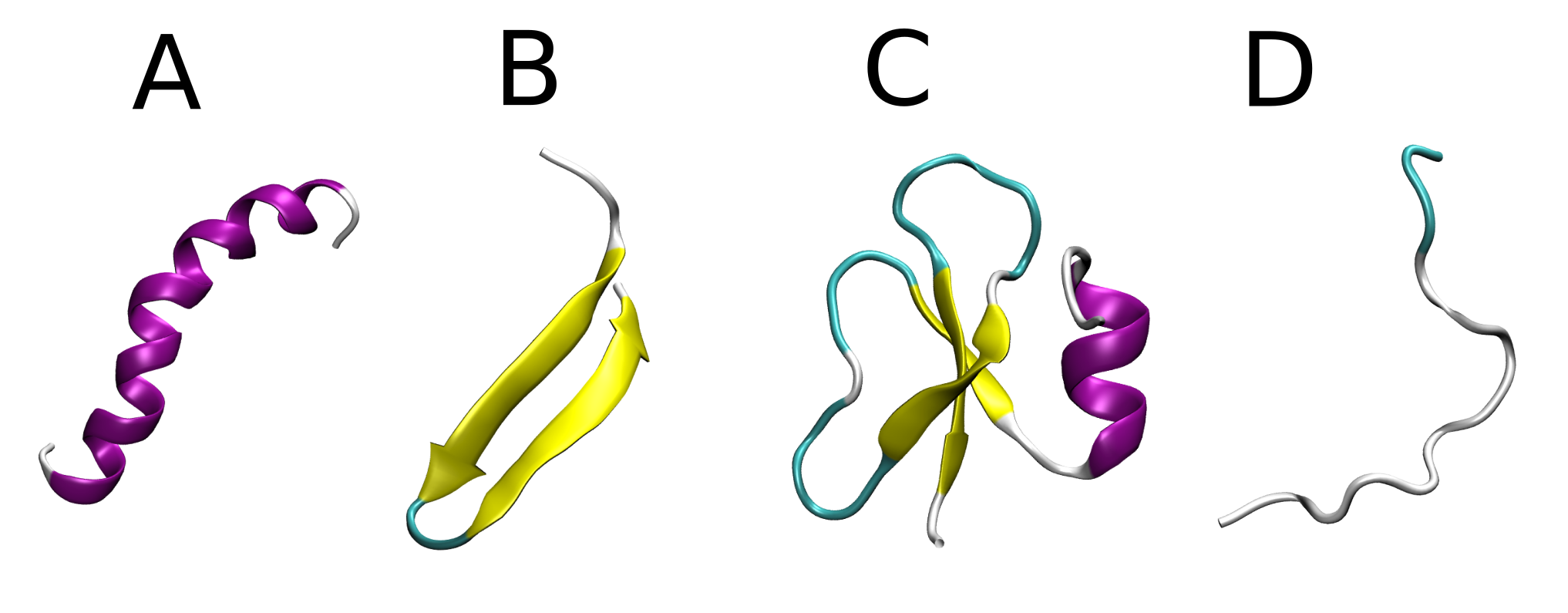


Figure 2: Common AMP folds, represented by example AMPs. A) α-helix (represented by melittin, PDB 6DST); B) anti-parallel β-sheets (represented by protegrin-1, PDB 1PG1); C) mixed helical and sheets (represented by human β-Defensin 2, PDB 1FD3); D) extended random coil (represented by indolicidin, PDB 1G89). Helical, β-sheet, turn and coil elements shown in pink, yellow, cyan and white, respectively. Images prepared with VMD 1.9.4a31.

One important aspect of AMP research is to consider the therapeutic index of the peptide (i.e. the ratio of antimicrobial activity relative to mammalian cytotoxicity). In nature, AMPs selectively target invading microbes without detrimentally interfering with host cells, and the reasons for this have been related to their structure and physicochemical properties23–25. For example, AMPs are often cationic, meaning their initial electrostatic interactions favour bacterial membranes, which usually contain a multitude of anionic phospholipids, over mammalian membranes, which are usually composed of zwitterionic lipids25. Additionally, there is a more significant transmembrane potential present in bacterial cells, which has been hypothesised to increase the ability of cationic peptides to insert and disrupt the membrane23. Furthermore, mammalian cell membranes have a greater concentration of cholesterol, which is likely to weaken the hydrophobic interactions with the peptide24. Despite these features, some AMPs have reported mammalian cytotoxicity through pore-forming, hemolytic, and apoptotic mechanisms24,26–28, suggesting that it is important to model and experimentally determine the interactions of AMPs with mammalian cells as well as with bacterial ones in order to develop them into attractive drug candidates.

AMPs have already been the subject of extensive research, and a few dozen have been tested in clinical trials including daptomycin and colistin which are FDA (Food and Drug Administration) approved drugs29. While peptide therapeutics have traditionally been employed against metabolic disorders and cancers30, AMPs find use against infectious diseases. However, experimental approaches to explore AMP activity31,32 are traditionally low throughput, and have benefited from computational studies33–37. These studies use statistical pattern recognition methods to mine and interrogate databases of identified AMPs38 and/or mechanistic methods, in order to understand the mechanisms of action of known AMPs: this can inform the rational design of novel AMPs.

Computational tools can give specific mechanistic insight and can process large amounts of sequences, yet are still fraught with unique challenges. For example, one issue with using machine learning approaches to predict AMP activity is that they require well-tested, rigorous mathematical models that rely on diverse and normalized datasets for training, and these are hard to acquire and assemble. Similarly, mechanistic studies of AMPs interacting with a membrane usually are able to only represent very small numbers of AMPs and small and simplified models of the membranes; these are limited by both lack of detailed knowledge as well as limitations in computational resources and the underlying methods. We have collected the peptides mentioned in this review in table 1 for easy reference.

Table 1: AMPs discussed throughout this review, amino acid sequences, charges, secondary structures in the membrane (if known) and associated references.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Sequence** | **Net Charge** | **Secondary Structure** | **PDB Entries** | **Refs.** |
| Gramicidin S | (dF)PVOL(dF)PVOL(cyclic) | +2 | Cyclic β-sheet |  | 10,11 |
| Dermcidin-1L (DCD-1L) | SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL | -2 | α-helix | 2NDK | 39–41 |
| Gloverin | DVTWDKNIGNGKVFGTLGQNDDGLFGKAGFKQQFFNDDRGKFEGQAYGTRVLGPAGGTTNFGGRLDWSDKNANAALDISKQIGGRPNLSASGAGVWDFDKNTRLSAGGSLSTMGRGKPDVGVHAQFQHDF | +3 | α-helix |  | 21,42 |
| Melittin | GIGAVLKVLTTGLPALISWIKRKRQQ-NH2 | +6 | Α-helix with central kink | 2MLT  6DST  6O4M  1BH1  2MW6 | 43–47 |
| SB1/ HB146 | KYKKALKKLAKLL-NH2 | +7 | α-helix | 6Q86  6Q79  (C-terminal fucosylated D-analogue) | 48,49 |
| Human β-Defensin 2 (HBD-2) | GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP | +7 | Antiparallel β-sheet and α-helix | 1FD3  1FD4  1FQQ | 50–52 |
| Maximin 3 | GIGGKILSGLKTALKGAAKELASTYLH | +3 | α-helix | 6HZ2 | 53,54 |
| LL-37 | LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES | +6 | α-helix | 2K6O  5NNM  5NMN  5NNK  5NNT  2FBS | 55–58 |
| PGLa | GMASKAGAIA GKIAKVALKAL-NH2 | +5 | α-helix |  | 59,60 |
| Magainin 2 | GIGKFLHSAKKFGKAFVGEIMNS | +3 | α-helix | 2MAG | 61–63 |
| Indolicidin | ILPWKWPWWPWRR-NH2 | +4 | Random coil/ turns | 1G89  1G8C | 64–66 |
| Alamethicin | Ac-(Aib)P(Aib)A(Aib)AQ(Aib)V(Aib)GL(Aib)PV(Aib)(Aib)EQ(Phl) | 0 | α-helix | 1AMT | 67,68 |
| Protegrin-1 | RGGRLCYCRRRFCVCVGR-NH2 | +7 | Antiparallel β-sheet | 1PG1  1ZY6 | 69–71 |
| Maculatin | GLFGVLAKVAAHVVPAIAEHF-NH2 | +4 | α-helix | 2MMJ  2MN9  2MN8  (G15 analogue) | 72,73 |
| Cecropin A-melittin (CAM) | KWKLFKKIGAVLKVL-NH2 | +6 |  |  | 74,75 |
| Phenol-soluble modulin α3 (PSMα3) | f-MEFVAKLFKFFKDLLGKFLGNN | +1 | α-helix | 5KGY | 76,77 |
| Temporin L | FVQWFSKFLGRIL-NH2 | +3 | α-helix | 6GS5 | 78,79 |
| MSI-594 | GIGKFLKKAKKGIGAVLKVLTTGL-NH2 | +7 | Helical-hairpin | 2K98 | 80 |
| Daptomycin | (dec)-WNDT(cyclic)GOD(dA)DG(dS)(MeG)U(cyclic) | -3 |  | 1XT7  1T5N  1T5M | 81–83 |
| human β-defensin 3 (hBD-3) | GIINTLQKYYC(1)RVRGGRC(2)AVLSC(3)LPKEEQIGKC(2)STRGRKC(1)C(3)RRKK | +11 | Antiparallel β-sheet and α-helix | 1KJ6 | 52 |
| LAH4 | KKALLALALHHLAHLALHLALALKKA-NH2 | +5 (neutral conditions)  +9 (acidic conditions) | α-helix | 2KJO (ph 6.1)  2KJN (ph 4.1) | 84,85 |
| Plectasin | GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY | +1 to +3, depending on pH | α-helix and two antiparallel β-sheets | 1ZFU | 86 |
| KLA-1 | KLALKLALKAWKAALKLA-NH2 | +5 | α-helix |  | 87 |

Despite these limitations, computational approaches are widely used to aid and complement AMP research. In this review, we will provide an overview of AMP research problems that benefited greatly from the use of computational tools; specifically, we have chosen to focus on computational studies that were conducted to probe the effect of AMPs on bacterial membranes The aim of this review is to collect relevant work in the field and provide a useful starting point and guide for computational modellers or for those seeking to complement experimental work on AMPs with computational tools. The first part of the review will cover methods for predicting the character and/or structure of novel potential AMPs; this involves pattern recognition and machine learning. The second part will focus on molecular simulation approaches that aim to elucidate the mode of action of AMPs by investigating their conformational and aggregation propensity and their interactions with model membranes.

# Approaches for Prediction of AMP Structure and Activity

Prediction approaches are used to discover novel sequences with AMP character, predict the mode of action or target of a known AMP, or to suggest a likely three-dimensional (3D) structure when no experimental conformational data is available for a peptide. They accomplish this by mining available databases of existing AMPs to compare with putative sequences, while structural information can be obtained by homology with motifs known to have AMP activity.

In this section, we will highlight several computational tools that are used to predict and analyse these properties, and the applications they have found in the field of AMPs.

### Databases of AMPs, AMP Design and Activity Prediction Tools

Perhaps the most important resource one can use to predict the AMP character of a novel sequence is a catalogue of existing AMPs, to be used as a reference and as a guide. To this end, there are several available databases listing known AMPs (natural and derived) that are annotated with relevant information. These sequences can be used to scan the literature for peptides with similar origins or modes of action, helping to establish a relationship between structure and desired function. Databases may include information on the peptide such as source organism, modifications, physico-chemical parameters, structure, putative mechanisms, and others; these tags and features make it easy to sort and identify relevant entries. Some of the largest databases are general in nature, but some more specialised ones focus on AMPs from the same type of organism, such as frogs or plants, or on AMPs of the same type, such as peptaibols or bacteriocins (Table 2).

Table 2: Publicly available databases of AMPs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Sequences** | **Structures** | **Kind** | **Website** | **Ref.** |
| CAMPR3 | 8164 | 757 | General | [camp.bicnirrh.res.in/](http://www.camp.bicnirrh.res.in/) | 88 |
| APD | 3165 | 409 | General | [aps.unmc.edu/AP/main.php](http://aps.unmc.edu/AP/main.php) | 89 |
| YADAMP | 2525 | 2525 (predicted) | General | [yadamp.unisa.it/about.aspx](http://yadamp.unisa.it/about.aspx) | 90 |
| PhytAMP | 271 | 39 | Plant AMP | [phytamp.pfba-lab-tun.org/main.php](http://phytamp.pfba-lab-tun.org/main.php) | 91 |
| DBAASP | 14496 | 1692 | General | [dbaasp.org/](https://dbaasp.org/) | 92–94 |
| MLAMP | 878 | No | General | [www.jci-bioinfo.cn/MLAMP](http://www.jci-bioinfo.cn/MLAMP) | 92 |
| ADAM | 7007 | 759 | General | [bioinformatics.cs.ntou.edu.tw/ADAM/index.html](http://bioinformatics.cs.ntou.edu.tw/ADAM/index.html) | 95 |
| DADP | 2571 | No | Frog | [split4.pmfst.hr/dadp](http://split4.pmfst.hr/dadp/) | 96 |
| Bactibase | 230 | 53 | Bacteriocins | [bactibase.hammamilab.org/main.php](http://bactibase.hammamilab.org/main.php) | 97 |
| Peptaibol Database | 317 | 7 | Peptaibols | [peptaibol.cryst.bbk.ac.uk/home.shtml](http://peptaibol.cryst.bbk.ac.uk/home.shtml) | 98 |
| DRAMP | 20227 | Present (statistics unavailable) | General | [dramp.cpu-bioinfor.org/](http://dramp.cpu-bioinfor.org/) | 99,100 |
| BAGEL | 814 | Present (statistics unavailable) | Bacteriocins | [bagel4.molgenrug.nl/index.php](http://bagel4.molgenrug.nl/index.php) | 101 |
| ADAPTABLE | >40,000 | Present (statistics unavailable) | General | <http://gec.u-picardie.fr/adaptable> | 102 |
| InverPEP | 774 | Description only, if it is known (statistics unavailable) | Invertebrates | https://ciencias.medellin.unal.edu.co/gruposdeinvestigacion/prospeccionydisenobiomoleculas/InverPep/public/home\_en | 103 |

These databases provide a vast collection of sequences that can be used to guide the rational design of AMPs, including optimising a known peptide, or generating a *de novo* sequence104. Peptide optimisation relies on understanding physico-chemical parameters, specifically those that affect the membrane-disrupting properties of many AMPs. Peptides that adopt α-helical conformations lend themselves well for this purpose, as the charge distribution, hydrophobicity and amphipathicity of helices correlate with membrane activity105. By understanding how individual amino acid mutations change those properties, researchers can modulate the AMP character of peptides, and generate new sequences. Dathe and al., for example, obtained several KLA-1 analogues by modifying the overall cationic charge of the peptide87 probing in this way the effect of the hydrophobic moment on hemolytic activity; they also obtained magainin II analogues with improved antimicrobial activity through mutations of the parent peptide that changed the overall charge and related properties106. Similarly, Giangaspero et al. investigated the effect of sequence length and chirality, among others, on a template helical AMP sequence107, optimising antimicrobial activity while balancing cytotoxicity. This sort of approach often makes use of helical wheel projections as a way to visualise the amphipathicity of a peptide. AMP sequence optimisation can also involve the use of non-natural amino acids105 and staples108. Generally speaking, optimising a sequence relies on “chemical intuition” and experiment, and *in silico* tools are often used to understand molecular modes of action, which are described in the second portion of this review.

Where computational methods truly shine in AMP design is in the identification of *de novo* sequences. Thanks to the availability of large datasets, researchers can build sophisticated models that tease out patterns and fundamental principles where the human eye would fail. These models train on thousands of known AMPs to infer relationships and correlations between sequence and properties, so that users may submit a putative sequence and obtain the likelihood of their sequence possessing antimicrobial activity; they can also try to predict the specific mode of action109 or the target organism110. Some developers of AMP databases offer this data-mining-based prediction as an extra feature, as do CAMPR388, ADAM95, or BAGEL4 (specifically for bacteriocins111), using machine learning methods that we will outline in the next section. Some web servers, generally using the databases mentioned above as a training set, provide a more streamlined approach if all one requires is a simple activity prediction: we have listed them in table 3. Each tool tends to claim higher accuracy than comparable methods, so meta-analyses of different approaches are valuable. A previous study has found that the CAMPR3 prediction tool generally performed the best112, although follow-up studies suggest that certain databases are more suited to more narrow tasks, such as predicting linear peptides or specific classes of AMPs113. A more recent comparison published in the last year by the developers of the AmpGram prediction tool114 naturally suggests that their own method is more accurate, but it should be noted that the different analysed predictors, which include CAMPR3, AMPScanner, ADAM, iAMPpred and iAMP-2L, have largely yielded high performances across different data sets. The most recent review of AMP prediction tools available in the literature115 reaches a similar conclusion, with several tools having comparable accuracy; the ones that performed the best, across multiple data sets, were amPEPpy116 and AMPfun117, although on a set of ten more recent AMPs, IAMPE118 had the highest correct prediction rate.

Table 3: Publicly available AMP activity prediction tools.

|  |  |  |
| --- | --- | --- |
| **Name** | **Website** | **Ref.** |
| AmpGram | http://biongram.biotech.uni.wroc.pl/AmpGram/ | 114 |
| AntiBP Server | [webs.iiitd.edu.in/raghava/antibp2/help.html](https://webs.iiitd.edu.in/raghava/antibp2/help.html) | 119 |
| AntiBP2 Server | [crdd.osdd.net/raghava/antibp/](http://crdd.osdd.net/raghava/antibp/) | 120 |
| Antimicrobial Peptide Scanner | https://www.dveltri.com/ascan/ | 121 |
| AMPA | http://tcoffee.crg.cat/apps/ampa/do | 122 |
| AMAP | <http://amap.pythonanywhere.com/> | 123 |
| iAMP-2L | <http://www.jci-bioinfo.cn/iAMP-2L> | 124 |
| iAMPpred | <http://cabgrid.res.in:8080/amppred/> | 110 |
| CS-AMPPred | https://sourceforge.net/projects/csamppred | 125 |
| amPEPpy | https://github.com/tlawrence3/amPEPpy | 116 |
| IAMPE | http://cbb1.ut.ac.ir/ | 118 |
| AMPfun | http://fdblab.csie.ncu.edu.tw/AMPfun/index.html | 117 |

Needless to say, the results of a prediction query should always be vetted and checked. Current methods, for example, may overestimate the AMP character of longer sequences112, a bias possibly caused by the sets of peptides used for their training, and have in general resulted in high rates of false positives115. When building a new model for the prediction of AMPs, we cannot stress enough the importance of non-AMPs in the training set, which act as true negatives104. Another area where current methods are largely lacking is in the prediction of functional activities115, which is the prediction of the specific kind of antimicrobial activity (antibacterial, antifungal, antiviral, etc), and is attempted by only a limited number of tools.

In the next section, we will detail the methodologies of some of these prediction tools, with a special focus on machine learning. It is one of the best available strategies for identifying patterns in large datasets, with many different implementations: these range from complex, resource-intensive models to simpler regression tools that even novices in the field can apply.

## A Brief Introduction to Machine Learning in the field of AMPs

Quantitative Structure-Activity Relationship (QSAR) modelling is a long-standing approach that aims to predict properties of chemicals by analysing the underlying molecular descriptors126. An example of this would be to predict the boiling point of a molecule by comparing its functional groups with those of molecules whose boiling point is known, and using this analysis to infer a relationship between structure and properties. In recent years, this approach has taken advantage of Machine learning (ML) algorithms, sometimes referred to as Artificial Intelligence (AI). These are computational tools that use statistics to find and learn patterns in massive amounts of data; specific implementations vary widely, but generally speaking ML tools use a portion of the available data, known as the training set, to establish which features correlate more strongly with the objective property, and then test that hypothesis on the rest of the data, called the test set. This kind of ML is known as supervised learning.

ML methods have found application in virtually every field even beyond chemistry, and AMPs are no exception: recent innovations in ML algorithms and the availability of better quality AMP datasets have enabled ML-aided prediction and design of AMP candidates127. ML applied to AMP sequences128 involves statistical learning to infer empirical relationships between amino acid composition and physico-chemical properties of peptides with antimicrobial activity. These models can be used to (a) predict AMP activity (binary classification) (b) predict the mode of action and (c) predict the efficacy of antimicrobial activity (multi-label classification model or regression). The amino acid sequences of peptides are annotated with associated physico-chemical properties, and these are used as feature vectors for training the algorithms. Depending on the availability of training data, the prediction models can be further tuned to understand sequence-activity relationships in AMPs and to design AMPs with enhanced potency and antimicrobial efficacy; methods of choice for prediction models include support vector machine (SVM), k-nearest neighbor algorithm (KNN), random forests (RFs), and artificial neural network (ANN), among others. We refer to table 4 for a summary of the more common ML methods that have been used in AMP research. These methods, as mentioned, try to find patterns in the AMP properties, and the most commonly used features for this purpose are peptide length, molecular weight, amino acid composition, charge, lipophilicity, and net charge, although they are by no means limited to these. Figure 3 shows a schematic representation of a typical ML workflow, applied to the field of AMPs. In the next few paragraphs we will highlight specific studies that have employed ML tools to investigate the AMP character of new sequences or to understand properties of known AMPs.

Table 4: Commonly used Machine Learning (ML) Methods in AMP Research.

|  |  |  |
| --- | --- | --- |
| **Method** | **Description** | **Reference** |
| Support Vector Machine (SVM) | Supervised learning algorithm that finds a hyperplane that satisfactorily separates known data in categories, and uses that to categorise new data. Accurate, less computationally demanding, and particularly well-suited to categorisation problems. | 129 |
| k-Nearest Neighbour (KNN) | Supervised learning algorithm that divides known data based on proximity. Easy to implement, but may scale poorly with larger data sets. | 130 |
| Random Forest (RF) | Ensemble learning algorithm that builds decision trees that categorise the data. Simple to implement and understand, but may struggle with larger data sets and may overfit if not careful. | 131 |
| Artificial Neural Network (ANN) | Supervised or unsupervised learning algorithm that uses interconnected nodes to process the data and find trends. Highly effective, but requires large and diverse datasets for training; it is comparatively more resource-intensive and results may be hard to interpret. | 132 |
| Hidden Markov Model (HMM) | Probabilistic method that finds attempts to infer a hidden variable from observed variables. Particularly well suited for sequential data, such as sequences of amino acids in a peptide. | 133 |

One of the first studies in this field was a QSAR AMP prediction tool based on ANN, SVM and quantitative matrix models119, using motifs found in the C- and N-terminal residues of peptides from the APD134 (Antimicrobial Peptide Database). The authors found that SVM, with an accuracy of 92.11%, performed better than the other two methods, whose accuracies were 88.17% (ANN) and 90.37% (quantitative matrix). Cherkasov et al., using an ANN model135 combined with multiple iterations of experimental data collection and model training, screened and identified 50-100 novel peptides with activity against drug resistant bacteria, mainly using *Pseudomonas aeruginosa* as a model but also *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterobacter cloacae*, and others. This was an early example of the possibilities of computer-aided rational design in the field of AMPs.

Another example of the early use of multi-label classifiers to predict the different modes of action and targets of AMPs was developed by Xiao et al.124 using a fuzzy KNN algorithm, named iAMP-2L; the 2L refers to 2 level predictors. Five main types of functionalities were predicted: antibacterial, anticancer, antifungal, antiHIV, antiviral. The authors achieved an accuracy of 86% in predicting whether a sequence was an AMP or not, while predicting what kind of functionality it had had an accuracy of ~60%. This was considered to be a good performance since there was little training data on various functional classes. The method used physico-chemical descriptors such as the hydrophobicity, molecular weight and isoelectric point of individual residues along with pseudo-amino acid composition notation124 to preserve sequence-order information.

More recently there have been some promising developments in the application of AMP prediction models with the application of SVM on secondary structural features along with the physico-chemical properties of peptides by Meher et al.110 The authors have additionally built source-organism specific AMP prediction models and obtained over 90% accuracy. This study also reported one of the highest numbers of AMP sequences used in an AMP prediction study, collecting 8913 sequences from various AMP-databases, including 5652 positive ones. The authors have developed a server called *i*AMPpred thatcomputes the probability associated with a given peptide sequence of being antibacterial, antiviral and/or antifungal. Another SVM model called CS-AMPPred125 predicts antimicrobial activity based on five descriptors of secondary structure and sequence properties, with ~90% accuracy. SVM is robust, accurate and less resource intensive compared to other ML methods, and hence is a popular choice in the field of AMP prediction136.

Another systematic attempt in the field of multi-label classification has been reported by Liu et al.109using a two-layer RF-classifier, yielding an accuracy of ~87%. The first layer predicts if a peptide is an AMP or not, and the second layer predicts the mode of action and target organism using a version of RF known as pruned set-RF. This study also used a larger feature set which employed properties like solvent accessibility, polarizability and normalised van der Waals volume along with simple physico-chemical properties. Polanco et al.137 also developed an ML scoring function based on a Hidden Markov model that relies on physico-chemical similarity rather than amino acid composition, and used it to identify a specific subclass of AMPs known as selective cationic amphipathic antibacterial peptides among known AMPs.

Neural networks have also seen recent application in the field of AMP prediction. Veltri et al.138 developed a deep neural network (DNN)that relies on a reduced amino acid alphabet that uses 9 categories instead of the 20 natural amino acids for simplifying experimental work, reducing the complexity of the available chemical space. The reduced alphabet eases the computational demands of the method while not compromising on accuracy. Another neural network method was used by Müller et al.139 by combining a recurrent neural network model (RNN) with a long short-term memory model (LSTM), a system already applied not only in protein secondary structure prediction and protein subcellular localisation prediction, but other fields outside of biology as well. Regarding AMPs, this method was applied in the prediction of amphipathicity as it is known to be a fundamental property140. The advantage of combining RNN and LSTM is that predictions made this way are “fuzzy”141, meaning that they do not merely replicate exact templates of the training data but can interpolate and find more subtle characteristics. However, this can come at the cost of being more resource intensive132. This method can be used to generate novel sequences with AMP properties, and it has a better predicted accuracy than randomly generating peptides.

There are still many more ML algorithms which we do not delve into, but which nonetheless find use in AMP research; these include naïve Bayes (NB)142, discriminant analysis143 and AdaBoost144, as well as others. The last two algorithms we want to highlight are n-grams and XGBoost. n-grams are used by AmpGram, hence the name, and are a concept used in computational linguistics to predict the relative probability of words appearing in a specific sequence, based to their overall probability in a reference data set145. In the context of AMPs, n-grams are used to predict amino acid motifs with AMP activity. This allows AMPGram to not only check the activity of novel peptides but also scan for so-called ‘cryptic’ AMPs, which are antimicrobial domains and segments of proteins that do not have antimicrobial activity and are obtained from proteolysis of the parent sequence146,147. For example, this approach has been used to find AMP-likely segments in the genome of chromatophores from the amoeba *Paulinella chromotophora*, in an effort to understand endosymbiosis and organellogenesis148. Finally, XGBoost145, which stands for eXtreme Gradient Boosting, is a relatively new ML algorithm. Gradient Boosting, the parent model, iteratively constructs weak predictors which become stronger as the ensemble grows; XGBoost is a highly optimised version of this process that focuses on efficiency. It is particularly well suited for classification and regression, and its main advantage is that it is very fast. The IAMPE predictor tool118 implemented several different ML strategies, and found XGBoost to be the best performing among the tested ones in terms of predictions. XGBoost has found applications in many fields beyond AMP research where it has proven to be a popular and effective algorithm149–152.

To conclude, the field of ML-assisted prediction of AMP sequences and activities is an ever-expanding one, augmented by the large number of available methods and models. These methods are not mutually exclusive; in fact, they are often used in conjunction to bolster each other and improve the quality of the results109,153. Simpler statistical tools are also available, such as logistic regression154. These methods can be used in many forms, and are available as open access libraries of scripts in packages such as scikit-learn for python155 or the mlr package for R156; amPEPpy, for example, employs a RF classifier using scikit-learn116, and IAMPE implemented five machine learning models (NB, KNN, SVM, RF and XGBoost) with scikit-learn. As our computational capabilities improve and we can perform more calculations faster, machine-aided prediction will play an increasing role in AMP research.

AMP Database

AMP Database

Training Data Set

AMP Database

ML Model

Prediction

Test Data Set

Evaluation

Feedback

Figure 3: A schematic representation of supervised ML methods in AMP prediction. AMP databases are a source of sequences with known AMP activity, which are collected in a training data set. It is important to carefully curate the data set, ensure diversity of the sequences and include true negatives, which in this case are sequences that do not have AMP activity. A poor training data set will bias the final results. This data is then used for the ML Model, of which we have described many implementations such as SVM, RF, ANN, and others. The model uses the data to find patterns and associate features with the chosen property, in this case AMP activity. The model provides a prediction of the combination of features that produces the intended behaviour. This prediction is evaluated with the test data set, a collection of sequences with known properties that was not used to train the model. If the prediction is sound, it will also properly categorise the test data set. The ML model can be then refined using the results of the evaluation.

### Secondary and Tertiary Structure Prediction

We have now reviewed how different methods can predict the AMP character of a novel peptide sequence, but this may be insufficient. As the function of a biomolecule is defined by its 3D structure (and associated dynamics in different environments), it is clear that the prediction of a property based on sequence alone is bound to be limited (unless the structural information is unambiguously encoded into some feature, which currently is not the case). Hence, the prediction of secondary and tertiary structures is crucial. The problem is exacerbated in this field because, as previously mentioned, only a small minority of AMPs have experimentally solved structures22, and thus it can be harder to determine an input sequence’s secondary or tertiary structure from comparison with known ones. This is problematic because some important properties, such as charge density, dipole moment and hydrophobic moment, cannot be calculated from sequence alone and require a 3D structure153,157. Assignment of a secondary structure to a sequence, therefore, is paramount to fully analyse any peptide, and it is an ongoing endeavour which includes methods such as homology modelling158, structural-alphabet approaches159, machine learning approaches160,161 and *ab initio* methods162. Explaining these general techniques is beyond the scope of this article; suffice to say that they have been used with success in aiding AMP research in regard to secondary structure prediction163–166 (Table 5).

Table 5: List of peptide secondary structure prediction tools.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Website** | **Notes** | **Ref** |
| PEP2D | [crdd.osdd.net/raghava/pep2d/](http://crdd.osdd.net/raghava/pep2d/) | Webserver | 167 |
| PEP-FOLD 3.5 | [mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3](https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3) | Webserver | 159 |
| PEPstrMOD | [osddlinux.osdd.net/raghava/pepstrmod/](http://osddlinux.osdd.net/raghava/pepstrmod/) | Webserver | 168 |
| Jpred 4 | [compbio.dundee.ac.uk/jpred/](http://www.compbio.dundee.ac.uk/jpred/) | Webserver | 169 |
| PHD | [npsa-prabi.ibcp.fr/cgi-bin/npsa\_automat.pl?page=/NPSA/npsa\_phd.html](https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_phd.html) | Webserver | 170 |
| PSIPRED | [bioinf.cs.ucl.ac.uk/psipred/](http://bioinf.cs.ucl.ac.uk/psipred/) | Webserver | 171 |
| SOPMA | [npsa-prabi.ibcp.fr/cgi-bin/npsa\_automat.pl?page=/NPSA/npsa\_sopma.html](https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) | Webserver | 172 |
| I-TASSER | https://zhanglab.ccmb.med.umich.edu/I-TASSER/ | Webserver | 173 |
| QUARK | https://zhanglab.ccmb.med.umich.edu/QUARK/ | Webserver | 174 |
| LOMETS | https://zhanglab.ccmb.med.umich.edu/LOMETS/ | Webserver | 175 |
| Robetta | [https://robetta.bakerlab.org](https://robetta.bakerlab.org/) | Webserver | 176,177 |
| MODELLER | https://salilab.org/modeller/download\_installation.html | Downloadable program | 178 |
| DeepCNF | [raptorx.uchicago.edu/download/](http://raptorx.uchicago.edu/download/) | Downloadable program | 160 |
| DNSS2 | [github.com/multicom-toolbox/DNSS2](https://github.com/multicom-toolbox/DNSS2) | Downloadable program | 179 |

There are also methodologies that are AMP-specific180, drawing from the previously mentioned databases of AMPs to identify common folds and structures, which can be used to assign secondary and tertiary structure to uncharacterised sequences in a more targeted manner. This approach relies on previous studies that have identified general conformations adopted by AMPs; broadly speaking, these can be distinguished based on adoption of α-helical or β-sheet secondary structures, extended structures rich in specific amino acids, loop conformations181, or combinations of these structures95 (Figure 2). Prediction is also carried out by matching new sequences with known ones to suggest potential secondary structures for the query182. This is a continuously evolving area with several challenges; for example, categorising AMP conformations by global secondary structure can lead to classifications that are too broad, and dihedral angles provide a more granular metric140. The ability to predict the folds and 3D structures of AMPs will improve as more tertiary structures are reported in the literature and smarter algorithms are developed140,183,184. Furthermore, modelling simulations of biomolecules require a starting 3D structure, which may be provided by crystallography or NMR studies, but, where those are unavailable, can be provided by secondary/tertiary structure prediction tools. In the likely case that a novel AMP sequence lacks an experimentally-obtained conformation, these tools can be used as a starting point for the mechanistic studies described in the second half of this review.

In summary, it is clear that regardless of the method, matching a sequence with its structure is of great importance towards gaining a mechanistic understanding of an AMP’s mode of action.

### Aggregation Propensity Prediction

The last category of tools we wish to discuss in this part of the review is that of aggregation propensity predictors. As we will explain more in detail in a later section, aggregation is the ability of certain species to form supramolecular structures with copies of itself, and it is an important parameter to consider when examining the properties of AMPs185. Knowing if an AMP is more or less likely to aggregate, as well as the properties of the aggregate, provides valuable insight on the mode of action of the AMP. For this purpose, there are several tools freely available online or as downloadable executables. These tools are not AMP-specific, and in fact are ‘borrowed’ from amyloid research, in which there is also interest in determining the aggregation propensity of peptides. They have however been employed in AMP research186,187, with TANGO usually the most popular tool for the purpose, followed by AGGRESCAN. We have catalogued these tools in table 6. These methods generally work by measuring physico-chemical parameters of the peptide as a whole, or by scanning for known aggregation-prone segments or structures, or by a combination of these approaches188. They are not without flaws, however189: while features such as amino acid composition can be reliably used as a predictor of aggregation propensity, the effects of other factors such as impurities and post-translational modifications are much harder to incorporate in models. A thorough analysis of the system is important to determine if there are unknowns that might affect aggregation.

Table 6: List of available aggregation propensity prediction tools.

|  |  |  |  |
| --- | --- | --- | --- |
| **Tool** | **Website** | **Ref.** | **Notes** |
| AGGRESCAN | [bioinf.uab.es/aggrescan/](http://bioinf.uab.es/aggrescan/) | 190 |  |
| AmyloidMutants | [amyloid.csail.mit.edu/](http://amyloid.csail.mit.edu/) | 191 |  |
| AMYLPRED2 | [aias.biol.uoa.gr/AMYLPRED2/](http://aias.biol.uoa.gr/AMYLPRED2/) | 192 | Needs registration |
| CamSol | [www-mvsoftware.ch.cam.ac.uk/index.php/camsolintrinsic](http://www-mvsoftware.ch.cam.ac.uk/index.php/camsolintrinsic) | 193 | Needs registration |
| ChemAGG | [admet.scbdd.com/ChemAGG/index/](http://admet.scbdd.com/ChemAGG/index/) | 194 |  |
| FoldAmyloid | [bioinfo.protres.ru/fold-amyloid/](http://bioinfo.protres.ru/fold-amyloid/) | 195 |  |
| MetAmyl | [metamyl.genouest.org/e107\_plugins/metamyl\_aggregation/db\_prediction\_meta.php](http://metamyl.genouest.org/e107_plugins/metamyl_aggregation/db_prediction_meta.php) | 196 |  |
| Net-CSSP | [cssp2.sookmyung.ac.kr/](http://cssp2.sookmyung.ac.kr/) | 197 |  |
| Pafig | [www.mobioinfor.cn/pafig/](http://www.mobioinfor.cn/pafig/) | 198 | Downloadable executable |
| Pasta 2.0 | [protein.bio.unipd.it/pasta2/](http://protein.bio.unipd.it/pasta2/) | 199 |  |
| SecStr | [athina.biol.uoa.gr/SecStr/](http://athina.biol.uoa.gr/SecStr/) | 200 |  |
| TANGO | [tango.crg.es/](http://tango.crg.es/) | 201 |  |
| Waltz | [waltz.switchlab.org/](http://waltz.switchlab.org/) | 202 |  |
| ZipperDB | [services.mbi.ucla.edu/zipperdb/](https://services.mbi.ucla.edu/zipperdb/) | 203 |  |

### Summary

A prospective study with the goal of identifying novel AMPs has many tools at its disposal. There are several available databases that collect and categorise known sequences for easy access, and some of them even come with validated prediction tools. If a more tailored approach is needed, a wealth of machine learning approaches can be applied easily thanks to open access packages, and there are many existing implementations of machine learning algorithms to draw inspiration from. Once a new sequence with potential AMP activity has been identified, prediction of its secondary and tertiary structures can further augment the predicted antimicrobial potential. The aggregation propensity, an important property for some AMPs, can also be predicted.

Novel AMPs can then be validated via experiment, and/or be used in simulation studies to gain mechanistic understanding of their mode of action. The next section will outline various methods and studies reported in the literature that have been used to simulate the structural dynamics of AMPs in order to elucidate and develop models of their modes of action.

# Approaches to simulate AMP structures, modes of action and properties

Most AMPs are thought to kill bacteria by selectively disrupting bacterial membranes, causing cell leakage and death, although there are also reports of AMPs that act through other modes, such as several AMPs that can disrupt bacterial DNA inside the cell204 and oncocin, that targets ribosomes intracellularly205. We have chosen to focus on membrane activity for this review, as the current consensus regards it as the prevalent mechanism206, and the computational approaches used to study it tend to be more complex due to the difficulty of incorporating lipid bilayers.

There is a number of underlying mechanisms speculated to be responsible for AMP activity on bacterial membranes, including the barrel-stave, toroidal and carpet mechanisms22 (Figure 1). Most mechanisms involve an initial step of the peptide being attracted to the membrane surface, driven by electrostatic interactions between the cationic amino acids of the peptide and the anionic phospholipid head groups, leading to an accumulation of the peptide on the outside of the membrane16,17,22. Once a critical concentration is reached, the peptides start to insert into and disrupt the membrane (Figure 1). It is clear that the presence of charged amino acids dictate which membranes the peptide is attracted to, while the hydrophobicity of the peptide likely determines its ability to penetrate into the hydrophobic membrane interior; the secondary structure and aggregation properties of the peptide may play a role in how it disrupts the membrane16,17,22. Understanding the mechanisms that underpin AMP behaviour may provide crucial guidance for the design of new, more-effective antimicrobials. In this section, we review the role of molecular dynamics (MD) simulations207,208 in the development of this understanding, including methods for studying peptide conformation, aggregation and interactions with the membrane.

MD is used to study the temporal evolution of the structural dynamics of the AMP in different environments using Newton’s laws of motion, based on the forces felt through bonded and non-bonded interactions, which are described by a force field potential209. Depending on the scale of the problem, explicit representations of all atoms (AA) can provide atomistic resolution210, while coarse-grained (CG) representations reduce the computational expense by grouping atoms into beads211. Understandably, AA simulations provide a more realistic interpretation of the underlying energy landscape, while CG simulations are less accurate but allow larger systems and longer simulation times to be reached212. In addition, there are several enhanced sampling techniques and advanced methods suited for more specific studies; we have tabulated relevant methods in table 7.

Table 7: Simulation techniques that are used to study AMP processes and properties discussed throughout this review.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Process/ property of interest** | **Simulation technique** | **Description** | **Considerations for AMPs** | **Refs.** |
| Peptide secondary structure | Conventional atomistic molecular dynamics (MD) | Calculation of the dynamic evolution of atoms based on a force field potential and Newton’s 2nd law of motion. | * Can be used to monitor conformational stability of experimental peptide structures in solution or interacting with membrane models * May not be able to capture slow folding events due to the long simulation times required to overcome the associated energy barriers * Multiple simulation repeats may be required to increase statistical significance | 49,53,209,213–216 |
| Replica exchange approaches | A range of enhanced sampling techniques used to increase the conformational sampling achieved by MD. Multiple replicas of the system are simulated in parallel, with increasingly scaled temperatures or interaction parameters. Higher order replicas can access more areas of the energy landscape, while the base replica samples from an unbiased canonical ensemble. Coordinates are exchanged between replicas based on an acceptance test, meaning that the base replica is able to “jump” over energy barriers into new minima. | * Can capture slower peptide conformational changes than conventional MD * Can be used to perform *ab initio* folding of peptides, meaning the choice of starting structure is less significant * Fewer simulation repeats are needed but it is still advisable to check that simulations starting from different conformations converge to the same point | 217–221 |
| Accelerated (a)MD | An enhanced sampling technique that involves applying a bias potential to modify the true potential every time the system drops below a given “boost energy” value. This increases the rate at which the system can escape from energy minima. | * Can capture slower peptide conformational changes than conventional MD * Can be used to perform *ab initio* folding of peptides, meaning the choice of starting structure is less significant * Fewer simulation repeats are needed but it is still advisable to check that simulations starting from different conformations converge to the same point | 222,223 |
| High-temperature (HT) MD | Conventional atomistic MD of peptide-bilayer systems at artificially high temperatures. | * Elevated temperatures can speed up the kinetics associated with peptide insertion and folding in the membrane * The thermodynamic minima of many membrane-associated helices have been shown to be unaffected due to the increased thermostability of peptides in the membrane * It may be necessary to check the thermostability of an AMP experimentally to validate results * It may also be necessary to check the ability of the chosen force field to function correctly at nonstandard temperatures | 219,224 |
| Peptide aggregation | Conventional atomistic MD | As described above | * Can be used to monitor the stability of experimentally-determined peptide aggregate structures * May not be able to capture slow aggregation events due to the complex energetic landscape * Multiple simulation repeats required for statistical convergence | 209,225–227 |
| Coarse-grained (CG) MD | Multiple atoms are grouped into interaction beads, which reduces the computational expense by reducing the number of calculations required and smoothening the energetic landscape. | * Larger systems and longer simulation times can be achieved, meaning aggregation events are more accessible * Atomic detail is lost, which could lead to important events being overlooked. For example the changes in peptide conformation that may occur during aggregation | 212,228,229 |
| Metadynamics | A history-dependent bias is added to the potential, with respect to a given reaction coordinate, throughout the simulation. This has the effect of pushing the system away from previously explored regions of the energy landscape. At the end of the simulation, the sum of the bias potential along the reaction coordinate can be accumulated to deduce the underlying energy landscape. | * The technique can be used to enhance the sampling of slow aggregation events that may not be accessible to conventional MD * The bias needs to be applied to appropriate reaction coordinates in order to capture the aggregation event * It can be hard to choose reaction coordinates if the oligomeric structure is unknown | 226,230,231 |
| Umbrella Sampling (US) | The system is restrained at different values (windows) of a given reaction coordinate and MD data are sampled. The data are then combined and the restraint bias is removed to calculate the underlying energy landscape with respect to the reaction coordinate. | * The technique can be used to enhance the sampling of slow aggregation events that may not be accessible to conventional MD * As with metadynamics, an appropriate reaction coordinate needs to be chosen to capture the aggregation event * This can be difficult if the oligomeric structure is unknown | 74,232 |
| Peptide-membrane interactions | Conventional atomistic MD | As described above | * Can be used to investigate the surface interactions of AMPs with lipid bilayers or the interactions of AMPs embedded within the bilayer * Can also be used to perform self-assembly simulations of peptide-bilayer systems, which eliminates the need to overcome the energy barriers associated with peptide-penetration into a preformed bilayer * Transitions between metastable states unlikely to occur due to the computational expense associated with overcoming energy barriers * Multiple simulation repeats may be required to achieve statistical significance | 209,233,234 |
| CG MD | As described above | * Larger systems and longer simulation times can be achieved, meaning peptide penetration into the bilayer and pore forming events are more accessible * Atomic detail is lost and important peptide-membrane interactions could be overlooked, such as the bidentate interactions that could occur between arginine side chains and phospholipid head groups | 212,235,236 |
| US | As described above | * US can be used with the reaction coordinate set as the centre of mass (COM) distance between a peptide and the membrane. This allows the sampling and free energy calculation of the peptide moving from bulk water into the centre of the membrane. * The COM distance may be an over-simplistic reaction coordinate to describe the AMP penetration/ pore-forming process * It may be difficult to choose a more appropriate reaction coordinate to capture the complex processes undertaken by AMPs | 232,234,237,238 |
| Metadynamics | As described above | * As with US, Metadynamics can be used to enhance the sampling of an AMP entering a lipid bilayer and provides a free energy surface along the given reaction coordinate * Again, the COM distance reaction coordinate may not capture the complex AMP penetration/ pore-forming process * It can be difficult to choose a more appropriate reaction coordinate if the exact AMP pore structure/ mechanism is unknown | 231,239 |
| HT-MD | As described above | * As described for peptide secondary structure, elevated simulation temperatures can speed up the kinetics involved in AMP insertion into the membrane and folding * Not only does this achieve peptide folding, it can also help identify AMP-induced pore structures in the membrane * Again, it may be necessary to check the experimental thermostability of the AMP of interest and the functionality of the force field at high temperatures | 224,240 |
| Electroporation | This method involves applying an artificial transmembrane potential to increase the tension in the bilayer, which increases the likelihood of forming a pore | * The technique forces poration of bilayers and can therefore be used to increases the sampling of AMPs entering pores * Once a pore is formed and AMPs are inserted, it is common to remove the potential and observe the stabilisation of the pore by the AMP | 241,242 |
| aMD | As described above | * As well as increasing the sampling of peptide conformations, the method can also be used to increase the sampling of the AMP interacting with a lipid bilayer * This is more efficient than conventional MD as the system should be able to escape energy minima quicker and access different metastable states | 222,223 |

### Simulating AMP Conformational Propensity

Peptides fold into various conformations based on their amino acid sequence and biological environment. It is known that many AMPs fold into an active conformation upon binding to (or insertion into) the membrane, as can be observed with, for example, melittin that adopts random coils in aqueous solution but forms an ɑ-helix upon binding to a lipid bilayer243. The preferred mechanism of interaction of an AMP or its aggregates with the membrane interface, followed by insertion, depends on the conformations accessible to the peptide. Experimental techniques commonly used to study the structure of AMPs in different environments include X-Ray crystallography for probing their crystalline forms49,244,245, solution-state NMR for probing their aqueous forms (or active forms in membrane-mimicking solvents53,216,246), solid-state NMR for their membrane-embedded forms247, and circular dichroism (CD) for their secondary structures in solution or embedded in the membrane216,243,248,249. Comprehensive reviews of the experimental techniques used for biophysical characterisation of AMPs are available250. These methods have been complemented extensively by MD simulations, examples of which will be discussed here.

X-Ray crystallography is widely used to obtain conformations of peptides and proteins49,217, but these static structures may not represent the more biologically relevant structures that the peptide adopt in the solution phase, due to desolvation and crystal packing effects. MD has been used to examine the solution-phase behaviour of AMPs and compare it to their X-Ray structures. For example, Baeriswyl *et al.* found that the helical structure of the AMP SB1 seen in crystals is unstable in solution, and performed conventional atomistic MD starting from a 4-helix bundle (representing the crystal form) and an isolated helix (representing the solution form) to observe the interactions and solvation effects responsible for this behaviour49. They determined that aggregation and bundle formation are crucial for stabilising the peptide, as well as the fundamental role of N-acylation in the peptide’s AMP activity. Furthermore, enhanced conformational sampling approaches, such as Hamiltonian replica exchange (HREMD), are increasingly being used to enable sufficient conformational sampling and was used to formulate a molecular explanation for the observed differences between the X-ray crystal structure and solution-phase NMR structure of the AMP Human β-defensin 2 (HBD-2)217.

One of the areas where MD simulations have made a significant contribution is in exploring the difference in conformational propensity of AMPs in solution and within membrane models, which is important due to the difficulty of experimentally determining membrane-phase behaviour of AMPs. This sort of investigation can be carried out by placing experimentally-determined or predicted peptide conformations above or within a membrane model and monitoring the ensuing structural changes during the MD simulation53,213–216. These simulations allow comparisons to be made about differences in interactions between the AMPs and different membrane compositions, and help determine the effect that the membrane environments have on the AMP structure. For example, it has been shown with conventional united atom MD that the presence of negatively charged lipids (characteristic of bacterial membranes) electrostatically stabilises the ɑ-helix of the positively charged AMP LL-37, and this is absent in the interactions with the zwitterionic mammalian membranes, thus providing a mechanism underlying the ability of the selective targeting of bacterial membranes by LL-37213. Similarly, conventional atomistic MD has been used to monitor the stability of the NMR-determined structure of Maximin 3, an AMP with known anti-HIV activity, by simulating it in a sodium dodecyl sulfate (SDS) micelle model that mimics the target membrane53. This revealed that the peptide resides at the micelle interface where the mix of electrostatic and hydrophobic interactions induces a more ordered ɑ-helix than that seen in solution-state NMR53. In this case, the use of MD in combination with NMR provided additional information about the structure of the peptide in the membrane environment.

Efforts have also been made to use MD as an *ab initio* peptide folding approach in order to predict AMP structures, which is useful when experimental data is unavailable218,219. To achieve peptide folding from an extended structure, it is important to implement approaches that enhance conformational sampling because the energy landscape associated with peptide folding is complex and conventional MD is unlikely to reach the timescales necessary to sufficiently sample the process230. HREMD is an enhanced sampling approach frequently used to sample peptide conformations and has proved useful in sampling melittin, an AMP derived from honey-bee venom, on the surface of a phospholipid bilayer, allowing observations to be made about how the structural propensity of the peptide changes as it interacts with the membrane218. For some helical membrane-active peptides, it has also been shown that elevated simulation temperatures can speed up the kinetics of membrane-insertion and peptide-folding without affecting the thermodynamic minima visited by the peptide within the membrane224, in a technique named high-temperature MD (HT-MD). This is possible because, as shown by Ulmschneider *et al.,* membranes can increase the thermostability of helices and prevent unfolding224. Chen *et al.* used simulation temperatures of up to 120ºC to study melittin interacting with a POPC bilayer, allowing the peptide to insert into the membrane and fold into its experimentally-known helical structure within 2 µs219.

A key issue to consider when performing peptide simulations is the choice of force field. Different force fields model the underlying atomic interactions in different ways and are parameterised based on different datasets, meaning that it is possible that two force fields will not produce the same conformational landscape for the same peptide218. For this reason, it is advisable to test the ability of various force fields in describing the AMP of interest by comparing simulations with experimental data, such as NMR and CD data, as well as to other force fields. Furthermore, it is crucial to note the importance of performing multiple simulation repeats when studying AMP structure, especially when using conventional atomistic MD that is known to struggle to overcome high peptide-folding energy barriers. This also highlights the necessity of starting conventional atomistic AMP simulations from experimental or predicted structures.

Finally, it is useful to note some important trajectory analysis methods used in the study of peptide conformations. In order to obtain meaningful information about the structures present throughout a simulation, it is necessary to define reaction coordinates that describe the property of interest. For example, peptide conformation can be characterised based on the φ and ψ backbone dihedral space251. Other coordinates used to define peptide conformations include radius of gyration252, root-mean-squared deviation of atomic positions (RMSD), number of intramolecular hydrogen bonds, residue contacts, helicity and dictionary of protein secondary structure (DSSP) analysis253. Once appropriate reaction coordinates are chosen, it is important to monitor their values throughout the simulation to check for convergence. The reaction coordinates can also be used by various clustering methods to identify significant peptide conformations present in the simulation254.

To summarise, there is a well-known relationship in biology between form and function, and AMPs make no exception. To understand their shape and structure is to gain insight into their behaviour and ability to interact with membranes. Although there are several spectroscopic techniques that can be used to obtain an experimentally validated structure, these may not be sufficient to obtain detailed mechanistic insights. In this section, we have outlined the computational methods that are commonly used in the literature to complement experimental techniques. Simulations have helped speculate on the solution-phase structures of AMPs and how these structures may change when they interact with membranes. These methods provide the starting structures used in the simulations that aim to understand more complex AMP behaviours, such as aggregation and membrane penetration. In the next sections, we will explain the methods through which these phenomena are studied.

### Understanding AMP Aggregation Propensity and its Role in AMP Activity

Peptide aggregation results when multiple copies of the same peptide accumulate by interacting and forming clumps or clusters255. One of the most well-known examples of peptide aggregation is in amyloidosis diseases, such as Alzheimer’s and Parkinson’s256, which are characterized by assemblies of misfolded proteins into amyloid structures; aggregation is also seen in AMPs257. Figure 4 shows in cartoon form some of the supramolecular assemblies that AMPs can form, and discusses their role in activity. Understanding when and how AMPs aggregate can shed light on their mode of action74,258, and there are several experimental techniques that can be used to probe this behaviour, including, but not limited to, Cryo-TEM259, small-angle scattering260 and dynamic light scattering261. In this endavour, computational techniques are often used in conjunction with experimental results to rationalise and predict observations262, offering atomistic-level insight that cannot be accessed by experiment alone. In this section, we present studies that have made good use of simulations to understand the aggregation behaviour of AMPs, and what are the main considerations and challenges for those who wish to venture into this field.

A key problem has been understanding the link between AMP aggregation and its ability to penetrate membranes, and associated events such as pore formation. Computational studies are unique here as they can be used to study varying parameters and conditions that would be impossible to control in a laboratory, such as the exact number of peptides that can interact with each other, their charge, the size of the membrane, and so on. This can lead to an atomistic understanding of what drives aggregation and how it relates to AMP activity. Sengupta *et al.* established through simulations that for the well-known AMP melittin, aggregation was an important prerequisite for pore formation225. It was previously reported that a stable helical secondary structure is the conformation with which melittin binds to membranes263, but this study suggested that such a structure is not required for pore formation, and in fact it might not be necessary for antimicrobial activity. Instead, the simulations propose that an increase in local concentration caused by aggregation enhanced the propensity to form pores in the membrane. A similar simulation study by Woo *et al.* also reached the conclusion that melittin and its mutants form pores through aggregation264. Likewise, Han *et al.* used simulations to investigate how two different AMPs, PGLa and magainin 2, synergistically aggregate together to penetrate bilayers228.

Oligomer

Vesicle

Covalent dimer

Fibril

Nanofibres

Liposaccharide aggregate

Individual AMP

Figure 4: A cartoon representation of various possible supramolecular structures available to AMPs through self-assembly185. Not all AMPs can access all structures: the formation of a structure is highly dependent on sequence, environment and conditions. Oligomers are aggregates of AMPs of variable size265, and can cause membrane destabilisation via curvature strain or the generation of grain boundaries. Cysteine-linked dimers have also been reported266, with possible increased antimicrobial activity and lowered toxicity. Vesicles267, nanofibres268 and amyloid-like fibrils269 are more complex self-assembly products that can impart additional properties such as improved delivery and hemocompatibility. Finally, AMPs can form assemblies with other molecules such as bacterial liposaccharides270, which can give rise to antimicrobial effects.

However, not all computational studies of AMP aggregation require the presence of a membrane, even if pore formation is the focus of the investigation. Marinova *et al.*, for example, note how computational AMP-bilayer studies may start from systems featuring only a single AMP interacting with a lipid bilayer184,240,271, but that may not be the form adopted when they interact with the membrane. Using indolicidin as a model AMP and employing CG simulations229, they investigated the behaviour of the peptide in solution, determining that it forms aggregates very quickly with an average size of 14 monomers per cluster, which could be even larger depending on the simulation conditions. This is an important observation, as it informs any further studies of the peptide with bilayers by determining the most likely structure adopted by the AMP when interacting with the membrane, and therefore the most adequate starting point for any such simulation. A similar study into the solution behaviour of melittin226 found that it preferentially forms tetramers in solution; these aggregates need to be considered when investigating membrane-bound conformations, as the presence of multiple peptides may affect their secondary structures243. These observations, however, need to be considered carefully, as the relationship between the behaviour in aqueous solution and the membrane-bound conformation may not be universal for all AMPs: for example, based on mathematical models272, alamethicin may be monomeric in solution and form aggregates only in the presence of membranes; protegrin-1 is known to form fibrils in solution273, but to interact with micelles as a dimer274. Further computational studies suggest that protegrin-1 needs to form structures comprising of 8 to 10 peptides to have AMP activity275.

There is also a host of enhanced sampling techniques reported in the literature that can be used to further, complement or aid studies of AMP aggregation, as well as integration with advanced experimental data. A simple and direct thermodynamic parameter to characterize the peptide aggregation propensity is the dimerization free energy (e.g., the potential of mean force), which has been extensively studied using MD simulations276,277. Moreover, information from experimental systems can be used to feed information useful for setting up more realistic simulations. Wang *et al.* monitored the leakage of fluorescent dyes from membranes in the presence of AMP maculatin using fluorescence spectroscopy, in order to estimate the number of peptides in a pore240, and this was subsequently used to guide the initiation of computational simulations. They discovered that maculatin can form several subtly different pore structures depending on the number of peptides in the aggregate, which may be an explanation for its resilience against bacterial AMP resistance. Similarly, Zou *et al.* have complemented experiments of magainin II bound with differing numbers of guanines with steered dynamics and umbrella sampling (US) simulations in order to determine the energy cost of embedding peptides into membranes74; these studies established a relationship between the number of bound guanines, aggregation propensity and antimicrobial activity. This relationship is hinted at in the literature for two other AMPs, PSMα3278 and Temporin L279, which suggests that the guanine modification might be a generalizable approach towards improving antimicrobial activity. Another enhanced sampling technique is metadynamics, which is used to “artificially” expand the potential energy landscape explored by the simulation, and was employed by Liao et al. to understand the pathways of aggregation for model AMP melittin, and study how these relate to membrane binding226.

A few words should be spent on discussing the best way to measure peptide aggregation computationally. In the examined studies, generally speaking, the two most commonly used metrics were the number of clusters present in the system and the number of peptides per cluster. A cluster is defined, in this context, as a complex of peptides of any size; for example, a system where peptides do not aggregate will have as many clusters as peptides, and in systems where they do, the size of the cluster (dimer, trimer, hexamer, etc) will be indicative of the extent of aggregation. The distance cutoff to describe a cluster may be dependent upon the analysed system but it has been reported to be typically in the range of 0.35280 to 0.5 nm, though generally not more than 0.8 nm. Another metric that can be used to monitor aggregate formation is solvent accessible surface area74: a decrease in this property can indicate that peptides are clustering. Time dependence is paramount in cluster analysis, as it can inform whether in a system the average cluster size has converged or it only forms transient aggregates; for example, studies have required up to 3 μs simulations just to reach equilibration228, but this is obviously highly dependent on the analysed system.

Further lessons on peptide aggregation can be learnt from studies of the aggregation of amyloid peptides, for which computational tools have been developed. These may involve monitoring the shape and predominant secondary structure of the cluster281, coarse-grained approaches as a method for examining large numbers of peptides at once282, and a focus on native and intermediate states to understand the aggregation pathways283. There have also been studies that have specifically looked at the interaction between amyloid aggregates and membranes284, which parallel efforts to understand how AMPs may disrupt bilayers. An alternative approach to understanding aggregation is to explore what disrupts it, as done by Iscen et al227 who used computational and experimental studies to explain how a Co(III) complex prevented amyloid aggregation, identifying the sites on the peptide that are necessary for self-interaction. In this case, hydrogen bond network analysis was used to define aggregation, coupled with secondary structure assignment. By understanding which residues are involved in aggregation, an AMP can be designed to promote clustering (if necessary for membrane interaction) or prevent unfruitful pathways (if specific aggregate structures do not lead to antimicrobial activity). As outlined in the prediction section, there are also tools that can predict the aggregation propensity of a peptide from its sequence alone.

What are, therefore, the key takeaways from studies of aggregation reported in the literature? There doesn’t appear to be a specific force field or computational package that is prevalent, as GROMACS, CHARMM and CG models are all used, often in conjunction with each other. As in other cases, the specific force field and what level of model is best is dependent on the system that is being analysed, and an appropriate model must be chosen carefully285. One thing was clear: multiple types of simulations were necessary to fully sample the complex landscape of aggregation, and to capture the mechanisms that lead from oligomerisation to pore formation and membrane disruption. In all of the referenced studies, simulations were repeated by varying the numbers of peptides, the lipid bilayer size, the length of the simulation, the presence of counter-ions, the charges on the peptide, and using peptides known not to have antimicrobial activity as negative controls. It is important to test different set-ups for these systems to identify the correct pathway and mode of action. Melittin and magainin 2, already amply used as model AMPs, are also studied extensively for aggregation purposes and can be an important point of reference. Aggregation behaviour can be an important metric to monitor, as knowing the most adequate size of clusters to use in peptide-bilayer simulations will lead to more accurate simulations. This can be understood by aqueous phase simulations of the peptide, or even by predicting the aggregation propensity from the sequence.

Aggregation is known to be an important mechanism for the antimicrobial activity of AMPs286. Whether the aim is to elucidate their mode of action, or to design new peptides287, it clearly is fundamental and can be explored with several computational tools and many different approaches.

### Peptide Effects on the Membrane: Insertion, Disruption and Pore Formation

The activity of many AMPs can be related to their ability to selectively disrupt bacterial cell membranes. While there exist experimental assays that can determine the presence of membrane disruption and AMP activity, such as cell or vesicle leakage assays288, it is difficult to experimentally determine atomic-level details of the exact interactions that occur between an AMP and its target membrane. Modelling and simulations have increasingly been used to explore such interactions and will be discussed in this section. Properties that are commonly used to measure these interactions include area per lipid, bilayer thickness, lipid chain order parameter, depth and orientation of the peptide in the membrane, lateral pressure, membrane curvature, clustering of charged lipids, electrostatic surface potential of the membrane, hydrogen bonds and electrostatic interactions between cationic residues with phosphate groups, free energy of peptide partitioning between the aqueous and membrane phases and presence of water deformations or water pores in the membranes289–291.

MD simulations have been used extensively in conjunction with experimental data to probe the structural dynamics of AMPs embedded in model membranes. In addition to probing the effect of the membrane on peptide structure, which was discussed previously, these simulations provide information about the effects of the peptide on the membrane and offer insight into the membrane-disruption mechanisms of AMPs . For example, the pore structures created in a membrane by β-barrel protegrin-1 oligomers were studied by simulating variations of oligomer-embedded phospholipid bilayers233. The study revealed important information about the AMP mechanism, such as the minimum number of monomers needed to keep a water pore open and the tendency of the β-barrel to tilt in the membrane in order to maximise favourable interaction233. Another study involved embedding MSI-594 into various lipid bilayers and performing accelerated atomistic MD, which resulted in more bilayer disruption in the bacterial than the mammalian membrane models; this is in agreement with the bacterial specificity of the peptide222. The embedding approach was also used to investigate the importance of bound Ca2+ ions on the ability of daptomycin tetramers to disrupt a bacterial membrane234. Monitoring the location and stability of tetramers in the membrane, along with the number of water molecules attracted into the bilayer, allowed critical comparisons to be made between the Ca2+-bound and the free tetramer234.

Some studies aim to sample full penetration and/or pore-forming events using advanced techniques that alter or speed up the sampling of the energy landscape in MD simulations, such as the previously mentioned accelerated atomistic MD. Simulation studies of membranes are computationally demanding, because they are large and require longer simulation time; employing CG helps obviate these problems212. Deng *et al.* used the MARTINI CG force field to simulate the long timescale behaviour of melittin and its variants interacting with a phospholipid bilayer235. In this study, several simulations were run to 10 µs (much longer than the timescales accessible to atomistic MD of similar sized systems), which was enough to observe multiple aggregation and pore-forming events. The study made several observations that were consistent with experimental data and the computational aggregation studies discussed above, including the need for the peptides to aggregate prior to pore formation and the relative pore-lifetimes induced by the different melittin variants235. The Dry MARTINI CG force field (where the solvent is represented implicitly) has also been used to investigate the effects of melittin on lipid vesicles236. Vesicle deformation and pore forming events were captured in just 1 µs, due to the increased sampling associated with the absence of explicit water particles. In the examples given above, CG simulations have allowed researchers to gain a broader understanding of the membrane-disruption mechanisms of melittin, proving that CG is a vital tool that can be used to corroborate observations about the bacterial membrane-disruption mechanisms of AMPs. A representation of how CG methods can be used to describe protein systems is shown in figure 5.

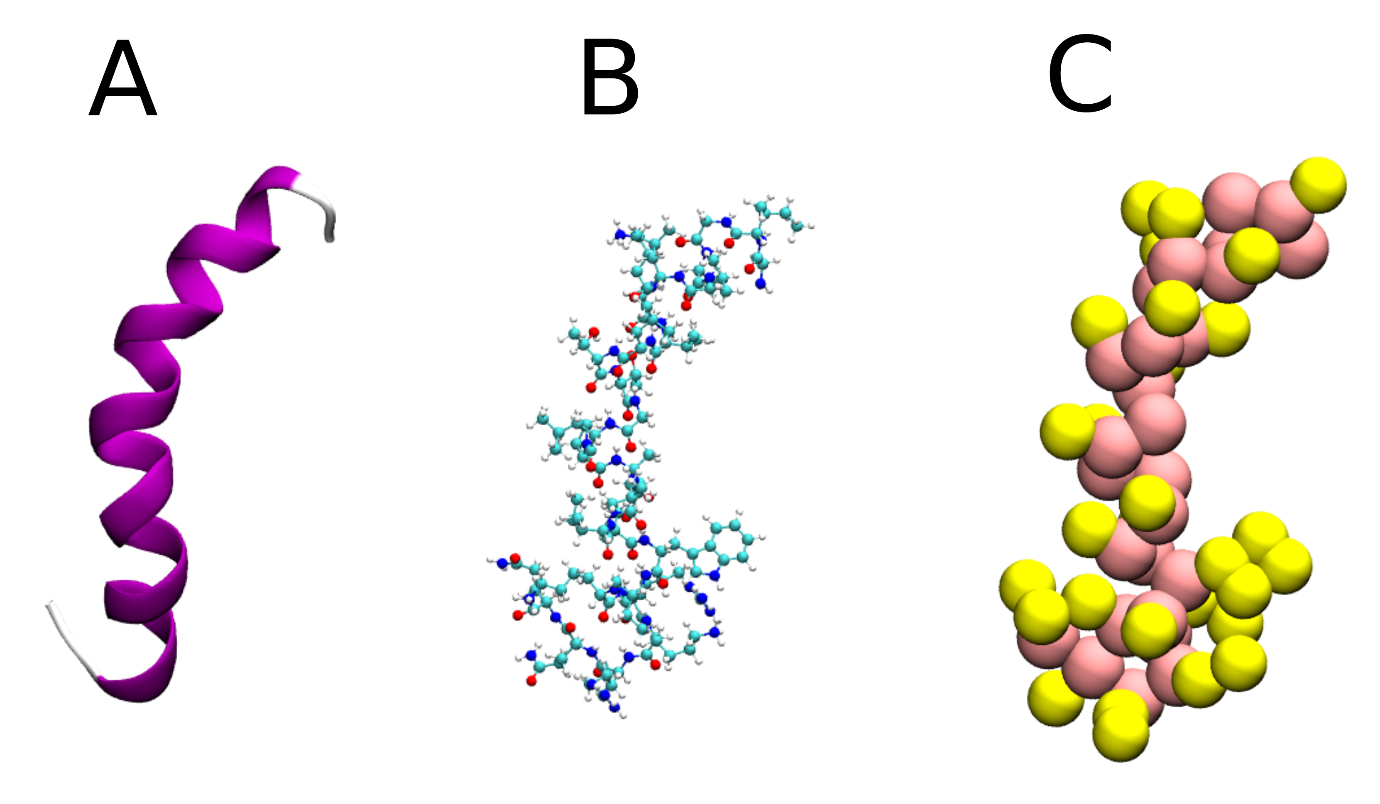


Figure 5: Melittin (PDB: 6DST) depicted as A) a secondary structure cartoon (helical and coil residues are depicted in pink and white respectively), B) an atomistic representation (using standard CPK atom colouring) and C) MARTINI coarse grained beads (backbone and side chain beads are depicted in peach and yellow respectively). There is a four-to-one mapping between the heavy atoms in the atomistic model and the beads in the MARTINI model. Images prepared with VMD 1.9.4a31.

Various other techniques also exist to increase sampling in the membrane. For example, umbrella sampling (US) is commonly used to study the free energy of moving a peptide from bulk water into a membrane234,237,238. Yeasmin *et al.* performed atomistic US simulations of human β-defensin 3 (hBD-3) penetrating through a lipid bilayer237. Comparing the energy surfaces created by a monomer and a dimer, along with properties such as bilayer thickness and peptide RMSD, allowed the authors to suggest that hBD-3 works as a dimer to induce a toroidal membrane pore237. Similar to US, metadynamics has been used to increase the sampling of AMPs moving through phospholipid bilayers while also providing details of the associated energy surface. Careful considerations however need to be taken into account as the membrane system is characterised by many hidden barriers, making the choice of appropriate collective variables less straightforward. Metadynamics has been utilised with the MARTINI CG force field to study melittin in a bilayer239. As introduced in a previous section, high-temperature MD may also speed up the sampling of AMPs in membranes by increasing the ability of the system to overcome kinetic barriers224. Wang *et al.* utilised this approach to study the ability of maculatin to form pores in a variety of membrane models by simulating multiple copies of the helical peptide on a bilayer at 353-373 K (80-120 ℃)240. They observed insertion and pore-forming events throughout the simulations and identified a variety of possible oligomeric pore structures. Finally, electroporation has been shown to increase the likelihood of membrane pores; this is a technique that involves simulating an artificial transmembrane potential to create tension in the bilayer241,242. The technique was used to study the translocation of protegrin-1 (PG-1) across a lipid bilayer by using a double bilayer system to create two water baths where the charge could be manipulated by removing and adding charged peptides or ions; the charge imbalance resulted in the observation of various peptide-lined pores, allowing suggestions to be made about the structure of PG-1-membrane pores241.

One potential limitation in classical MD simulations is the lack of incorporation of pKa shifts that occurs when a peptide is transferred from the aqueous phase to the membrane phase (e.g, the lipid tail region of the membrane). This is important because there is debate regarding the protonation states of amino acids in the bilayer, which greatly affect interactions with membranes; arginine was shown to be protonated in the bilayer center, but lysine is likely to be deprotonated at the bilayer centre292,293. As both arginine and lysine allow the peptide to form favorable electrostatic interactions with the membrane, it is clear that pKa shifts need to be accounted for. Developments of constant pH MD are being used to address these issues294,295, and has been applied in the study of AMPs. For example, Pohl et al. studied the pH-dependence of aggregation in the AMP plectasin296, determining that the peptide aggregated more at higher pHs. Similarly, Nhan et al. simulated how the structure of lactoferrin, a protein with antimicrobial activity from which AMPs can be extracted297, changes when deviating from neutral pH, and how these structural changes affect its antimicrobial properties298.

In order to study the preferred position and orientation of AMPs in lipid bilayers without biasing the outcome by choosing initial configurations, some studies have utilised a bilayer self-assembly approach, which involves placing the lipid, peptide and water molecules in a random configuration and observing the assembly of a peptide-embedded bilayer299–301. This technique allows the derivation of possible AMP pore structures without having to overcome the energy barriers associated with penetrating and permeabilising a pre-formed bilayer. For example, the Martini CG force field was used to simulate the self-assembly of fungal cell membrane lipids with a model β-sheet AMP with sequence RMCKTPCGKFYCYKPCP, resulting in the observation of semi-toroidal pores. Self-assembly was also used to study the differences between the protonated and unprotonated forms of LAH4, which is known to act as an AMP in acidic conditions but as a CPP in basic conditions, revealing that the protonated peptide had a greater impact on bilayer stability208,301.

Apart from bacterial cytoplasmic membranes, considerable efforts have also been focused on developing other model membranes, such as fungal membranes and the outer membranes of various types of Gram negative bacteria at both the atomistic and coarse-grained levels302–309. We have collected membranes discussed in this review in table 8. MD simulations of these membranes provide useful insights into the action mechanisms of certain AMPs. Li et al. used a four-component model membrane (POPC:POPE:POPS:ergosterol) to mimic the fungal membrane, and found that a branched peptide tetramer B4010 can preferentially interact with the anionic lipid POPS302. Subsequently, the same group studied the mode of interaction of a branched AMP dimer B2088 with the lipid A membrane, and observed substantial calcium release from the membrane surface and significant membrane deformation310. Similar membrane effects were also observed in the simulations of polymyxin B with the lipid A membrane311. Development of various types of model membranes greatly advances the computational understanding of the effect of AMPs on these membranes using MD simulations, such as the work done in the Khalid group which encompasses atomistic studies of bacterial membranes242, CG studies312 and updating existing force fields to more easily model membranes313.

Table 8: Lipid compositions used in the simulation papers discussed throughout this review and the membranes they represent. POPE: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; POPG: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1’-rac-glycerol); SDS: Sodium dodecyl sulfate; POPC: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; POPS: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine; POPI: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoinositol; DMPC: 1,2-Dimyristoylphosphatidylcholine; DMPG: 1,2-Dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol).

|  |  |  |
| --- | --- | --- |
| **Lipid composition** | **Membranes the model has been used to represent** | **References** |
| POPE:POPG 3:1 | Bacterial, Gram-negative, inner-leaflet | 74,214,216,222,241 |
| SDS Micelle | Bacterial | 53 |
| POPC | Mammalian, outer-leaflet | 222,238 |
| POPG | Bacterial, Gram-positive | 213,214 |
| POPC:POPG 3:1 | Bacterial, Gram-positive | 215 |
| POPE:POPG 7:3 | Bacterial | 233 |
| POPE:POPG:Cholesterol 7:2.2:0.8 | Bacterial | 233 |
| POPS | Mammalian, inner-leaflet | 222 |
| POPC:POPG 7:3 | Bacterial, inner-leaflet | 222 |
| POPC:POPS 7:3 | Bacterial | 222 |
| POPG:POPE 3:1 | Bacterial, Gram-positive | 222 |
| DMPC:DMPG 1:1 | Bacterial | 234 |
| DMPC | Mammalian | 234 |
| DOPC:DOPG 7:3 | Bacterial | 236 |
| POPE:POPG 1:1 | Bacterial | 238 |
| POPC:POPE:POPS:POPI 5:3:1:1 | Fungal | 299 |

To summarise, computational modelling and simulations are of great importance in aiding our understanding of how AMPs insert, disrupt and/or form pores in bacterial cell membranes. Not only do they provide atomistic detail that would be unattainable by experimental means, they also enable the fine tuning of system properties to probe their importance for AMP activity, such as altering lipid ratios to represent the membranes of different organisms (Table 2). However, as discussed with regards to peptide folding and aggregation, peptide-membrane simulations are subject to the challenges associated with force field accuracy and sampling. This is particularly true for lipid molecules, which are harder to accurately describe with force fields. A force field can only be validated by comparison with experimental work, and accurate experimental data about lipid bilayers is harder to obtain than for proteins, especially for biological bilayers that might include many different kinds of lipids. This makes deriving lipid force fields quite challenging, and extreme care must be taken when utilising them314. It has been shown that a “best” force field that works equally well under all circumstances cannot be recommended, so it is paramount to test different force fields for the desired system and choose the one best suited to the research question and properties of interest289. While conventional atomistic MD can be used to validate experimentally derived or predicted pore structures233, it is unlikely to reach the timescales required to observe spontaneous transitions between the various metastable states that may be important to the AMP mechanism. This highlights the importance of running multiple simulation repeats to increase the statistical significance of the observations. In addition, various enhanced sampling approaches can be employed to enhance the sampling of the system and shorten the time necessary to obtain meaningful results.

# Conclusion

AMPs represent a new, attractive class of drugs that is the subject of extensive research. They are finding increasing use against disease-causing microorganisms, which is a critical function as many of those organisms have developed resistance against commonly used antimicrobials. AMPs are being developed as drugs and several are in clinical trials, but more research must be done to understand their activity. Experimental work in the field can encounter limitations and challenges, which can be overcome by complementing such studies with computational methods. As we have seen, computational techniques have been used to predict the AMP character of novel sequences, which helps narrow down the search space in testing and alleviates the workload. This can be used to optimise existing compounds or generate new leads. We have also seen a plethora of simulation techniques that can elucidate and propose mechanisms and modes of action by investigating the atomistic interactions and dynamics of relevant biological systems.

However, although the field has attracted considerable attention in recent years, there are still issues that need to be resolved; ideally, prediction methods rely on robust, large and diverse data sets, but current databases lack sufficient negative and positive controls, contain data from multiple sources, varying experimental conditions and lack of generality as some peptides are only active against certain bacterial strains. Machine learning methods are only as good as the data used to train and test the models, and therefore their reliability is limited by the quality of the data sets. MD simulations depend on the accuracy of force fields and models, which always need careful refinement. They also encounter issues with convergence and sampling due to the computational expense associated with simulating large biological systems for the timescales necessary to observe the relevant processes.

Nevertheless, there is a considerable body of work that has explored the use of computational techniques in AMP research, and has been used, after appropriate benchmarking against experimental data, to design novel peptides that have been validated experimentally. This review can provide a starting point for computational AMP studies by summarising the key considerations involved in this area of research. Depending on the aim, a number of computational tools and methods are available and have been used to great effect. We hope this proves a useful guide for those attempting AMP research.

# Data and Software Availability

Software, web servers and computational tools described in this review are owned by their respective developers and copyright-holders. We have catalogued and reviewed them and have noted their accessibility, especially taking care of providing links if available, but can make no guarantees to their functionalities. Where we could not provide links, we encourage to contact the original authors of the relevant papers to obtain more information on the software and how to access it.

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