



Designing Antimicrobial Peptide: Current Status

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1. Introduction

Antimicrobial peptides (AMPs) are critical part of the human natural immunity, innate specifically, which is found among all modules of life. These peptides, which are produced by leukocytes, are working as broad-spectrum antibiotics that exhibit potential as novel therapeutic agents. AMPs have been confirmed to eliminate G^{-ve} and G^{+ve} bacteria, enveloped viruses, fungi, and even cancerous cells¹. AMPs eliminate these invading objects by direct antimicrobial effect as well as other effects like suppressing bacterial protective biofilm formation and enhancing the bacterial elimination by phagocytosis². AMPs, comparing to conventional antibiotics, may also have the ability to enhance immunity by functioning as immuno modulator through a variety of actions, like apoptotic control on immune cells, chemokine release by epithelial cells, enhancing wound healing, and many other actions that help promoting adaptive immunity³⁻⁴.

Replenishing the antimicrobial options is considered an urgent clinical need. In the past years, conventional antibiotics growth has gradually deteriorated, only two novel antibiotics have been permitted for public use in the past 8

years, and the latest novel class, daptomycin, was introduced thirteen years ago⁵. Multidrug-resistant (MDR) microorganisms are progressively predominant with a death rate reaching fifty-percent⁶. An important feature of AMPs over conventional antibiotics is that widespread resistance has not been reported. The resistance against conventional antibiotics is mainly due to limited number macromolecular targets, but it is hard to develop against AMPs since they usually work by attacking multiple cellular targets at the same time⁷⁻⁸. This feature is making AMPs the logical starting point for antimicrobial drug design. Nevertheless, AMPs resistance is a developing phenomenon, which is cancelling the effects and benefits of such peptides. Thus, in order to develop more powerful AMPs, it is a must to comprehend the complex mechanisms of AMPs resistance⁹ (Figure 1).

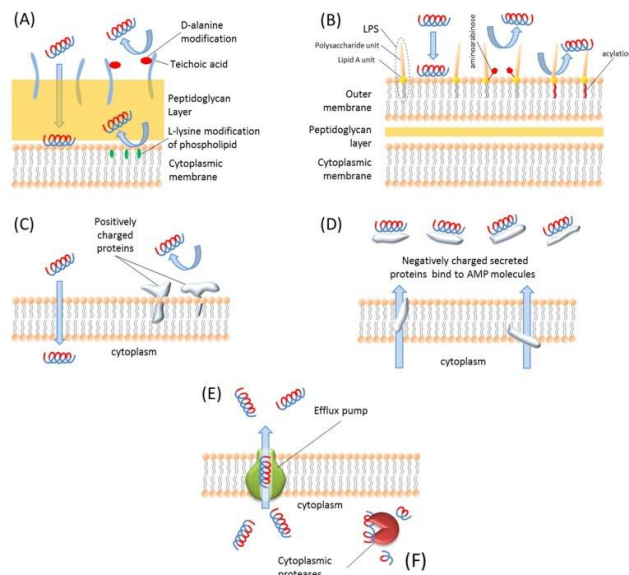


Figure 1. AMP resistance mechanisms: (A) Gram-positive bacteria resist AMPs via teichoic acid modification of LPS molecules and l-lysine modification of phospholipids. (B) Gram-negative bacteria resist AMPs by modifying LPS molecules with aminoarabinose or acylation of Lipid A unit of LPS molecules. (C) Bacteria express some positively charged proteins and integrate them in the membrane so positive charges repulse each other and bacteria can resist such AMPs. (D) Bacteria produce negatively charged proteins and secrete them into extracellular environment to bind and block AMPs. (E) The intracellular AMPs are extruded by efflux pumps. (F) The AMPs inside the cell are degraded by proteases. Adapted from “Antimicrobial peptides,” by Bahar A. A. & ren D. Pharmaceuticals. 6, 1543–1575 (2013).

Based on the 2nd structure, AMPs can be divided into four groups: 1) Alpha- helical; 2) Beta-sheet; 3) Mixed of both; and 4) extended¹⁰. Furthermore, AMPs bear both hydrophilic and hydrophobic parts that resulted from the separation of the polar and hydrophobic parts to different ‘sides’ of the particle in the functional structure¹¹. Although, the antimicrobial activity of AMPs was originally suggested to happen by distributing the membrane as explained by few models (barrel stave, toroidal, and carpet) (Figure 2), it has become clear that other mechanisms are involved like penetration into bacterial cytoplasm through targeting some membrane bond proteins¹². Furthermore, the latest

studies imply dual mechanisms of well-known membrane-disruptive AMPs including other actions like apoptosis induction¹³. For instance, magainin 2, which is well known membrane disruptive AMP by pore formation¹⁴, was recently discovered as having antimicrobial activity against *Escherichia coli* by inducing a mechanism similar to caspase-dependent eukaryotic apoptosis¹⁵. Although being explanatory for many aspects of antimicrobial activities, these mechanisms have not covered all ways of AMPs-bilayer interactions, the significance of each one of them, or even clarifying the importance of membrane binding for AMP selectivity¹⁶. Further lipids-peptides modeling analyses is necessary to accomplish significant progress in the design process based on interactions of the lipid membrane¹⁷.

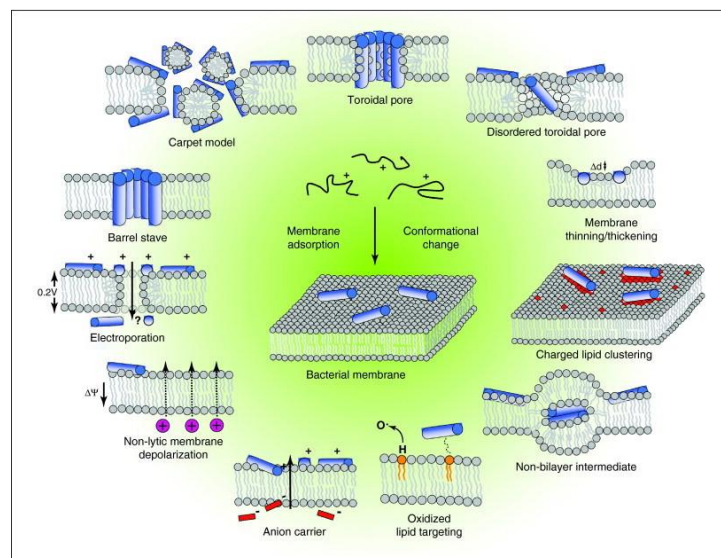


Figure 2. Events occurring at the bacterial cytoplasmic membrane following initial antimicrobial peptide (AMP) adsorption. Adapted from “The expanding scope of antimicrobial peptide structures and their modes of action,” by Nguyen LT, Haney EF, Vogel HJ, 2011, Trends Biotechnology: 29:464–72.

Despite being potent and effective compounds, AMPs have some drawbacks including limited antimicrobial action against certain germs species and serious side effects profile. For instance, horseshoe crab polyphemusin I, which is considered one of the most potent peptides against

bacteria and fungi, has no protective effect in neutropenic mice against *P. aeruginosa* and causes hemolysis at higher concentration¹⁸. Moreover, the phase three clinical trials of a Protegrin's derivative was suspended because it is leading to oral mucositis¹⁹. It is clear that the AMPs pioneering is costly and time consuming in using both *in vivo* and *in vitro* approaches and involve high risk side effect profile in *in vivo* studies. Therefore, to overcome these limitations, AMPs design using *in-silico* approaches is considered as a favorable method, which considerably lowers the production cost and the time needed for activity and toxicity evaluation. Due to their importance, *in-silico* approaches will be discussed in details through this review.

2. AMPs Databases

AMPs databases play a crucial role facilitating the design process since they offer scientists the opportunity to access sequences and structure data for tremendous number of AMPs from a broad range of organisms. New AMPs information, like any other entity, used to be accessible through journals articles and book chapters only. These methods became ineffectual due to the rapid increase in the number of discovered AMPs in the past several years. Since the launch of the first version of the Antimicrobial Sequence Database (AMSDb) at 1997 and until 2010, at least thirteen databases have been built for AMPs, which aid managing information, data explanation, AMPs analysis, and even prediction of AMPs from genomes not yet annotated¹⁷⁻²⁰.

AMPs databases can be classified into three main categories: i) general databases for naturally occurring AMPs (AMSDb, ANTIMIC, APD, CAMP, DAMPD, and YADAMP); ii) specialized databases for naturally occurring AMPs (Peptaibol, Penbase, Cybase, defensins, AMPer, BACTIBASE, and Phytamp); iii) specialized databases for non-naturally occurring AMPs (SAPD, RAPD, and BAAMPS)^{20,37}. In terms of quantity, these databases have progressed from a simple reservoir for relatively limited number and

manually collected AMPs to huge databases. For example, almost two thousand and seven hundred antimicrobial peptides are included in the new APD₃ database alone. Also, they have been improving in quality by using advance algorithmic tools for clustering and prediction. For instance, hidden Markov models have been utilized in AMPer database²¹ to predict novel AMPs in the bovine genome. Also, these models have been implemented in CAMP_{R3} database²² to work as family-based sequence signature helping to identify AMPs from a large pool of sequence data. Furthermore, highly diverse and comprehensive databases like APD₃, which covers six kingdoms including bacteria archaea, protists, fungi, plants, and animals, improved the diversity of AMP with pharmacological potentials²³.

It is in determinate if AMPs studies, which widely unrelated in terms of structure and function, will be helpful for understanding AMP activity in general¹⁷. This issue is giving us another reason to shed the light on the *in silico* approaches, which will help improving the databases by unraveling the underlying structure–activity relationships (SARs)¹⁷. Actually, effective synthetic AMPs have already been designed by Knowledge-based computational approaches. For instance, Adepantin 1, which has been designed using these approaches, has a potent antibacterial activity against and a higher selectivity for *E. coli* than the best AMP present in the AMP ad database based on therapeutic index²⁴. Furthermore, these approaches are helping predicting AMPs using support vector machine pair wise algorithm, which help predicting AMPs with sensitivity up to ninety-five percent from unidentified peptide sequences²⁵.

3. Physiochemical properties to consider when designing new synthetic AMPs

We do not have data demonstrating a possible association between the AMP structural domain and its mechanism of action, host range, or activity²⁶. Structurally similar AMP scan have enormously is similar types of targeted cells and

mechanisms²⁷. Though prediction of AMP activity and behavior based on structure alone may be unfeasible, certain general design parameters are important to follow and altering these parameters may help achieving such predictions²⁶. The ultimate objectives of this processes is to yield AMPs that are microbiologically effective and have lower side effect profile at desired therapeutic dose¹⁷.

3.1. Length

The length is vital factor for AMP activity because in order to produce amphipathic structure, you need at least eight amino acids in the sequence²⁶. Twenty-two and eight amino acids are required, for α -helical and β -sheet AMPs, respectively, in the barrel-stave model to transverse the lipid bilayer of bacteria²⁸. Furthermore, AMP length can affect its cytotoxicity. For instance, a shorter derivative of hp (2-20), if we compare it to the original form, is extremely less toxic to human red blood cells. Thus, when the goal is to design a low toxic AMP, the length of the sequence must be taken into consideration.

3.2. Net Charge

For a certain peptide, the total charge of the ionizable groups is considered the net charge. In case of AMPs, the charge varies from positive to negative and considers the main drive for the early contact with the cell membranes²⁶. Altering the net charge can change antimicrobial and hemolytic activities for certain AMP. For example, decreasing antimicrobial peptide L-V13K net charge eliminates its activity against *P. aeruginosa*, while increasing its positive net charge leads to severe hemolysis³⁰.

3.3. Helicity

Comparing to other factors, helicity is less important for antimicrobial activity of AMPs. Nevertheless, it is key determinant for eukaryotic cells toxicity³¹. Actually, reducing AMP helicity through D-amino acids integration into the main peptide sequence lowers the hemolysis, while

reserving the effect of antimicrobial³². Thus, D-amino acids incorporation is a helpful approach for designing novel synthetic peptides causing less hemolysis. Another important issue is the main sequence's amino acids tendency to form a helix structure. For example, proline has to be spared when designing α -helical AMPs because it is low helix-forming tendencies compared to other amino acids²⁶.

3.4. Hydrophobicity and Amphipathicity

Hydrophobicity influences both the AMP activity and selectivity, and about half of amino acids in natural AMPs sequences are hydrophobic residues³³. Reducing hydrophobicity can decrease antimicrobial activity, whereas increasing hydrophobicity on AMP positively charged side could surge its antimicrobial activity^{34, 31}. Also, hydrophobicity has a role defining the variety of target cells of an AMP. For instance, magainin, which is only has an action against G-ve bacteria in the wild form, has certain synthetic analogs that are effective against G-ve bacteria³⁵. Therefore, hydrophobicity must be taken into consideration when designing novel synthetic peptides.

Amphipathicity is an extra important feature ensuring the AMPs-membrane interaction. Despite being important for membrane-interface binding, the relative importance of amphipathicity to simple hydrophobicity-driven partitioning is unknown. *Fernandes-Vidal et al.*, studied this feature using interfacial partitioning and the results showed the superiority of amphipathicity on hydrophobicity for membrane binding³⁶. Thus, when designing AMPs for definite target microorganisms, primacy must be given to the amphipathicity feature of the structure.

4. *In silico* de-novo design of synthetic AMPs

The focus of current AMPs research is based on approaches to search, empirically or computationally, across the collection of identified or anticipated peptide sequences with desired properties¹⁷. Having the capability to analyze biophysical features from multiple points of view,

multi-scale approaches (statistical-based design strategies and molecular dynamics simulation) progressively applied to *in silico* enterprise of biologically active molecules³⁷. These approaches contain two main stages. First, *coarse graining* offers a quick evaluation of the objective space. Then, a transformed, more comprehensive demonstration of the coarse grain is produced to clarify each aspect of the biological process³⁷.

4.1. AMPs design and prediction based on statistical approaches

AMPs design and prediction based on statistical approaches are usually effective for speedy and accurate screening. In this design approach, a dataset of experimentally validated sequences is gathered to infer an adequate number of selected quantitative or qualitative features representing the desired behavior while topological descriptors account for sequence order information and secondary structure. Based on present knowledge, each amino acid sequence activity information in the database is coded to different types of variables. Then, an algorithm or regression is used to discriminate qualitative or quantitative peptide activities³⁷.

4.1.1. Dataset preparation

Throughout this step, an AMPs list, sharing a specific 1^{ry} and/or 2^{ry} structure information, is collected in a dataset. Because AMPs have remarkably diverse features, a comprehensive dataset is hard to obtain without introducing biases. In order to control those confounders, datasets that are manually edited are more appreciated²⁰. Preparing datasets by Ad-hoc approaches gives accurate and uniform information of the sequence behavior required by complex models³⁸. Although high-throughput screening of huge dataset is trending, systematic studies tend to reduce the number of screened peptides by analyzing a fixed combination of amino acids positions because high number of combinations makes comprehensive datasets screening unfeasible³⁹. One of main problems when

constructing an active and inactive AMPs data set is the undesirable addition of active peptides to the inactive peptide dataset when using a random selection approach from identified proteins datasets. In order to control this problem, knowledge-based approaches, like using Gene Ontology (GO) annotations, can be used⁴⁰. Another problem is data set compromising due to over-representation of certain sequence combination. To solve the problem, over-representative sequences have to be pruned from the datasets. For example, CD-HIT, which is an algorithm that trims out similar sequences, has been implemented and used in many datasets⁴¹.

4.1.2. Peptide representation

Each peptide needs to be presented based on its conformation since amino acids are considered the basic unit of AMPs³⁷. Linguistic models, where we consider sequences as 'words' and amino acid as 'one-letter', considered the simplest way to present AMPs sequences, where recurrences and grammar rules are used to estimate the significance of particular amino acid kind and location to peptide activity³⁷. Early linguistics models do not consider certain factors like amino acid position-specific interactions and physicochemical variables influencing peptides activity. These factors have been taken into account in the evolved forms of these grammar models by using different strategies to introduce secondary structure information like position-specific scoring matrix (PSSM)⁴². However, linguistic models are restricted to natural amino acids.

Quantitative Structure-Activity Relationship (QSAR) models have been used to handle these limitations through using the descriptors, which describe the association between biological activity and physicochemical characteristics⁴³. Molecular properties- such as polarity- can be described using global descriptors, while structure information - such as sequence order - can be described using topological descriptors. During the AMP design process, the choice of a particular descriptor is influenced directly by AMP

mechanism of action (Figure 3). For example, peptides that possess a balanced combination of hydrophobicity and +ve net charge are able to achieve sufficient antimicrobial effects because these descriptors are linked to invasion of the bacterial cell wall³⁷. Auto and Cross-Covariance (ACC) analysis is used to examine the correlation between the primary amino acid sequence and QSAR descriptors⁴⁴. The variables produced by ACC explain the distant interactions between amino acids along the peptide sequence⁴⁵.

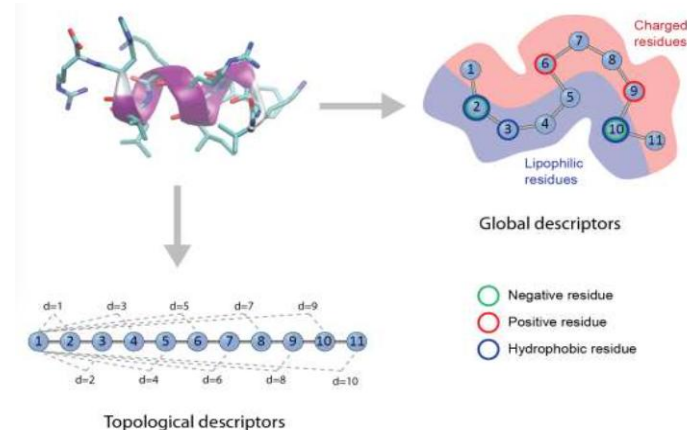


Figure 3. Representation of Feature selection process. Global and topological features are selected in order to represent the overall chemical physical characteristics and their distribution, respectively. Adapted from “In silico design of antimicrobial peptides,” by G. Maccari, M. Di Luca, R. Nifosì. *Methods Mol. Biol.*, 1268 (2015), pp. 195–219.

4.1.3. Prediction models

Two main categories of models can be distinguished based on biological activity information available in the data set: regression models for quantitative data and classification models for qualitative data³⁷. The choice between the 2 models involves a trade-off between model accuracy and meaningfulness. Because of their simple interpretation, linear models are widely used in AMPs design. For example, Support Vector Machine (SVM) is used to predict AMPs²⁵. On the other hand, artificial neural networks (ANN) offers better classification results, but at the expense of using relatively cloudy models that unable to discover the

underlying mechanisms involved³⁷. Decision trees are another method used for the classification. Random Forest (RF) considered the best decision tree to use in biological data mining due to their superior prediction accuracy and their ability to provide variable importance information, which is needed for a proper classification⁴⁶, with performances comparable to SVM and ANN⁴². (Table 1)

Algorithm	MCC		Prediction accuracy for test dataset (%)		
	Training dataset	Test dataset	Overall	Positive dataset	Negative dataset
DA	0.75	0.74	87.5	87.8	87.4
RF	0.86	0.86	93.2	89.9	95.4
SVM	0.88	0.82	91.5	88.0	93.8

***Table 1.** Performance of prediction algorithms. Adapted from “CAMP: a useful resource for research on antimicrobial peptides,” by Thomas S, Karnik S, Barai RS, Jayaraman VK, Idicula-Thomas S, 2010, *Nucleic Acids Res* 38:D774–80.

Although normalization of the descriptor set is required after choosing the statistical model to improve the accuracy of the model, some classification models do not require a normalization phase. In general, RF is capable of handling highly varying variables comparing to ANN and SVM³⁷. Another concern is eliminating unneeded descriptors since they affect the model functioning. A lesser quantity of descriptors is better, since the resulting model is simpler and less computationally expensive. Iterative methods like Incremental Feature Selection (IFS), or automatic methods, such as genetic algorithms (GA) can be used as a descriptors selection procedure⁴⁷⁻⁴⁸.

4.1.4. In silico sequence screening

Once that model is constructed, an automatic technique for a quick and efficient design and optimization has to be implemented. Stochastic methods of optimization, like Ant Colony Optimization or Genetic Algorithms (GA), have to be used since deterministic approaches would be impracticable due to the enormous amino acidic combinations needed to be explored⁴⁹. Specifically, GAs represent a multipurpose powerful tool for AMP design, since they follow the

environment adaptive approach³⁷. Every possible AMP candidate is considered like a certain population object, which will be fitted in a statistical model in order to reflect AMP biological activity. A certain number of random sequences are generated at the beginning of the selection process with increasing average fitness value for populations as the simulation goes on³⁷. Simultaneous optimization of objective, like the sequence length, by Multi Objective Evolutional Algorithms (MOEA) is required sometimes in the design resulted in a number of possible solutions being screened without preferring one particular objective⁵⁰.

4.2. Molecular Dynamics simulations of AMPs

Generally, biophysical principles are used to examine peptides interactions, process of folding and action mode for a selected list of peptides. Molecular Dynamics (MD) simulations, in particular, have been significantly used to study AMPs to explain the molecular mechanisms supporting their activity⁵¹ (Figure 4). In Force Fields based MD simulations, forces fields are called those defined by the coordinates and flexible mathematical functions terms, including covalent interactions (e.g. dihedral torsion) and non-bonded interactions (e.g. electrostatics)³⁷. MD simulations are making a noticeable progress clarifying peptide-bilayer interactions mechanisms⁵². Nevertheless, the results should be validated using experimental data due to the empirical nature of force fields and their lack of “utter” prediction accuracy³⁷.

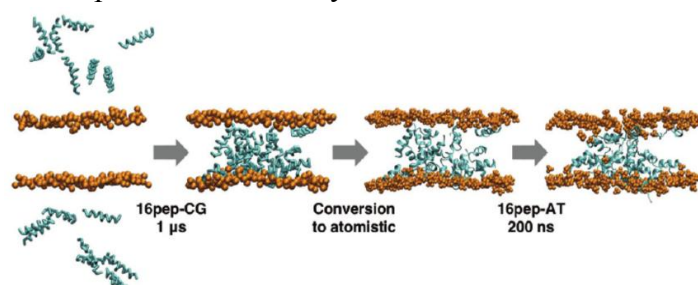


Figure 4. Multiscale Simulations of the Antimicrobial Peptide Maculatin 1.1: Water Permeation through Disordered Aggregates. Available from: https://www.researchgate.net/publication/221879297_Multiscale_Simulations_of_the_Antimicrobial_Peptide_Maculatin_1.1_Water_Permeation_through_Disordered_Aggregates [accessed Feb 2, 2017]

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4.2.1. Force Fields and sampling techniques

AMBER, CHARMM, GROMOS, and OPLS are force fields commonly used for simulation of AMPs⁵³. Essentially, each one of them contains numerous forms of an original force field created by a shared parameterization strategy³⁷. Therefore, each form might include different parameterization procedures, extension to different molecules, or modification of certain torsion terms. For example, Charmm36 includes the lipid force fields, whereas Charmm²⁷ includes the protein and nucleic acid force fields. Strengths and limitations of several diverse force fields applied to peptide simulations have been examined by validation studies, which proved that the late forms are superior at replicating a chain of experimental findings such as formation of beta-hairpin⁵⁴.

Resolutions ranging from atom level to diverse grades of coarse grain, which is packed units of atoms, can be used in MD simulations. However, all-atoms force fields use is limited due to incapability of present computational resources³⁷. Coarse graining the force fields is an effective strategy to speediness the process by decreasing the number of degrees of freedom in the model and it has been widely used for proteins and lipids that is applicable to peptide/bilayer simulations⁵⁵. The drawbacks of this approach are that secondary structure change cannot be simulated, and the over-estimation of pore formation energy requirement⁵⁶. Since it is switchable between coarse grain to all-atom, multi-scale methods can be implemented to handle such drawbacks⁵⁷.

Other techniques, alongside coarse graining, have been developed to overcome the restricted conformational space in MD simulations. These techniques might trace the relevant conformation states by utilizing collective variables like Umbrella Sampling and, more advanced approach, Metadynamics⁵⁸⁻⁵⁹. Meta dynamics solve the

problem of expanded number of needed simulations when performing multi-dimensional Umbrella Sampling by performing simulations where the system is “inhibited” to search the identical free-energy regions using memory of free-energy regions potentials⁵⁹. Nevertheless, a common problem with both approaches that they assume the time spent in the free energy surface is more than the time of relaxation in the same model⁶⁰. Another technique, which deals with this problem, accelerates the passage of free-energy barrier by coupling with higher temperature simulations like replica exchange⁶¹. Generally, all techniques mentioned above can be coupled together to ensure sufficient sampling of coordinates and degrees of freedom fast relaxation³⁷.

4.2.2. Systems and processes

Water, organic solvents, micelles, and lipid bilayer are different environments can be used by MD simulations to predict structural properties of AMPs. With suitable degrees of confidence and standardized pressure and temperature, structure predictions of peptides in solvents are possible³⁷. Water-organic solvent mixtures are utilized to evaluate the structure in various medias. For instance, water-2-trifluoroethanol mixtures media simulation, which provides a media somewhat, imitating the bilayer conditions, has been used to support new AMP sequences designing⁶². Micelles environment provides more accurate model of the lipid bilayer media due to fast relaxation time between solvent and lipid bilayer⁶³.

Since AMP inclusion in lipid bilayer is an extremely time consuming procedure for all-atom simulations, enhanced sampling techniques or coarse-grained force fields must to be used³⁷. Therefore, a different approach consists in starting from initial configuration where a random mix of lipids-water, which form a bilayer in 10-100 nano seconds, are used. Self-assembly of the lipid bilayer is simulated in a way that peptides position is not biased, during the self-assembly process,

toward the starting configuration because of the high fluidity of the system⁶⁴. Nevertheless, a more ambitious goal is simulating many peptides aggregation in the bilayer. The aggregates AMP may be pre-assembled to observe their steadiness and behavior, or the self-assembly method might be followed in the bilayer simulation. For example, AMP may be inserted at bilayer unbiased position and pore formation be observed⁶⁵⁻⁶⁶.

5. Modification of known AMP sequences (Templates)

The main aim of the modification studies is the alteration of known AMP sequences (templates) - like magainin-to improve antimicrobial activity or reduce toxicity⁶⁷. Despite their role in revealing the significance of specific amino acids to the sequence behavior, these studies do not account for the amino acid interactions influencing the global 3D conformation of the peptide⁶⁸. Nevertheless, we will go through some of the strategies of these modifications and their impact on templates structure.

5.1. Covalent Bonds

Antimicrobial effect of an AMP is modifiable when even changing a single disulfide bond. For instance, adding disulphide bond led to higher antimicrobial activities in sakacin, while removing disulphide bond led to inactivation of Protegrin against herpes simplex virus⁶⁹⁻⁷⁰. In another study, modifying the structure of indolicidin by adding disulfide bond and Trp-Trpcross linked to superior stability of protease without affecting AMP activity⁷¹.

5.2. Changing amino acid content

This is one of the most researched methods of modification, where the focus is on particular amino acids with physiochemical characteristics that are important to the activity and target range of AMPs²⁶. For instance, the effect of AMP proline content on their ability to penetrate cell membranes may due to low proline's tendency to

form alpha-helices. Higher proline content founds to reduce CP-26 ability to penetrate the cell membrane of *E. coli*⁷². AMP cytotoxicity can be affected by amino acids content changing. For example, the new synthetic LL37, which was modified replacing neutral with positively charged amino acids, showed lower cytotoxic effects on human cells, and then has been used effectively against *Methicillin-Resistant Staphylococcus Aureus* nasal infections⁷³.

5.3. Amidation

Incorporating of amide group in the structure of AMP is one of newest approaches of modifications. For example, a PMAP-23 modified with carboxyl end amidation found to have almost 10 folds faster G-ve bacteria membrane interaction, and more cellular uptake than the original form⁷⁴. In another side, de-amination has showed effect on AMP stability. Replacing Api88 C-terminal amide group with a free acid resulted in a fifteen times more stability against proteases in blood serum with no effect on the antimicrobial activity⁷⁵.

6. Conclusion

The obstacles facing conventional antibiotics, especially drug resistance and the steady decline in production and development, increased the need to look for an alternative. AMPs are considered an excellent alternative for conventional antibiotics due to their low probability of being resisted by microbes and their ability to enhance immunity by functioning as immuno modulators. However, controlling infections by AMP is still delayed due to several challenges including ineffectiveness against certain microorganisms and potential toxicity to eukaryotic cells.

Two main approaches to develop AMPs have been discussed in this review: modification of known AMP sequences and multi-scale *in silico* de-novo design of synthetic AMPs. Although they helped linking some peptide activities to amino acid type and position, modification of known

AMP sequences failed to explain the interactions between different amino acids and their influence on AMP tertiary structure, have high production costs, and they consume a lot of time to develop. On the other hand, multi-scale *in silico* approaches enable the identification of sequences in a time effective manner, have lower production costs, provide a comprehensive understanding of the peptide structural behavior in different environments (especially environments mimicking bio-membranes), and improve AMPs databases. Thus, utilizing *in silico* approaches has the potential to improve and accelerate AMP development. By accelerating the development process, additional AMPs might enter clinical testing and used as cures in the near future.

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