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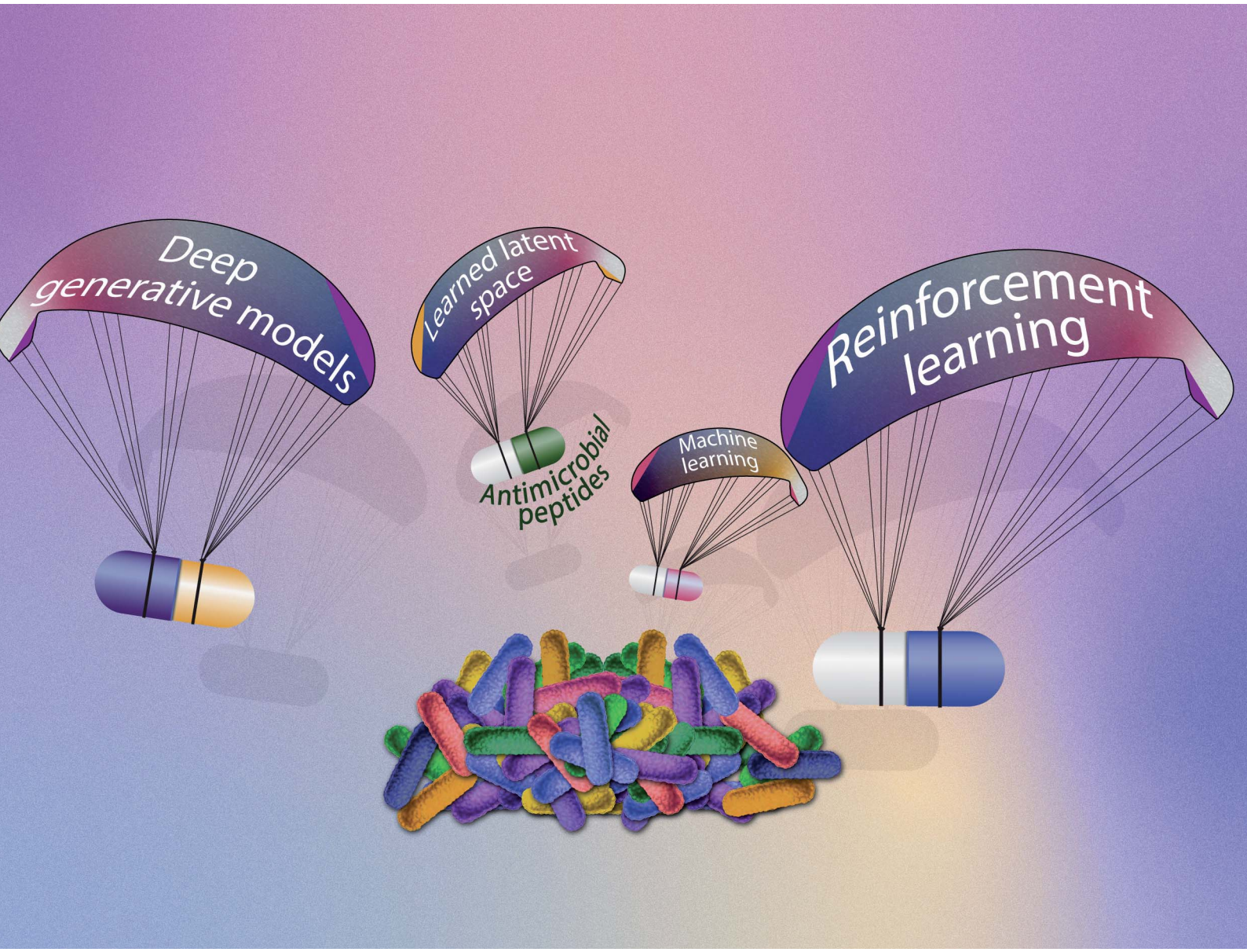

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PERSPECTIVE

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Progress and future of the computational design of antimicrobial peptides (AMPs): bio-inspired functional molecules

Miroslava Nedyalkova, *[ac] Andrew S. Paluch, [b] Diana Potes Vecini[ac] and Marco Lattuada *[a]

The effectiveness of antibiotics is greatly enhanced by their ability to target invasive organisms involved in the ancient evolutionary battle between hosts and pathogens. Conventional antibiotics no longer offer adequate protection due to the evolution of strategies to evade them. As a result, efforts are needed to design novel replacement antibiotics, making them unique from most other forms of drug development. As drug discovery costs have steadily increased along with the need for novel antibiotics, the interest in antimicrobial peptides (AMPs) as alternative antimicrobial treatments has grown in recent years. As a complement to experimental high-throughput screening, computational methods have become essential in hit and lead discovery in pharmaceutical research. It may be possible to access unexplored chemical space with customized virtual compound libraries. It has been questioned whether screening billions of molecules virtually with the risk of false positives is practical despite their unlimited potential size. In terms of finding novel chemical compounds capable of solving many global problems, machine learning, deep learning, and generative models hold significant promise. It is anticipated that the current challenges and limitations about the applicability of the stated approaches will be overcome in the coming years. However, plenty of advances are still required to achieve their full potential. In this perspective, we review the previous and ongoing work based on the latest scientific breakthroughs and technologies that could offer new opportunities and alternative strategies for developing novel AMPs.

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Introduction

Motivation

Pathogens resistant to conventional antibiotics have emerged and spread rapidly, increasing difficult-to-treat infections that threaten global health. By 2050, drug-resistant pathogens are predicted to account for the highest number of deaths worldwide due to infections.[1] Due to poor economic incentives and market failure, discovery and development efforts have gradually declined, and few new antibiotics have been commercialized in recent decades.[2] It is imperative that this trend be reversed with new strategies to develop novel antimicrobial treatments.

In terms of antimicrobial potential, metal complexes are an unexploded source. For instance, Rhenium complexes are particularly attractive given their low *in vivo* toxicity and high antimicrobial activity. However, their targets and mechanism of action need further research.[3–5] Microbial metabolites are the source of many existing antibiotics and other medicines. For their high diversity and broad bioactivity spectra, short peptides are among the most widely studied secondary metabolites, and the large group of antimicrobial peptides (AMPs) produced by bacteria has been used to treat bacterial, fungal, and viral infections and even cancer.[6] Previously, antibiotics were developed from bacterial antimicrobial peptides (AMPs), primarily non-ribosomally synthesized peptides and ribosomally synthesized and post-translationally modified peptides. Furthermore, class II and class III bacteriocins can be synthesized ribosomally and function unmodified.[7] As a result, they can be directly identified from microbial genomes, like AMPs found in eukaryotic genomes, such as human LL37 (cathelicidin).[8] In contrast to conventional small-molecule antibiotics, AMPs exhibit lower susceptibility to developing resistance in pathogens and encounter stronger phylogenetic barriers inside bacteria against horizontal transmission of developed resistance. While AMPs are diverse in amino acid sequence, they share several similarities, such as cationic charge (+1 to +7) generated by the presence of arginine/lysine/histidine residues, short amino acid sequences (frequently up to 50 residues in length), and are usually amphiphilic.[9,10] With contribution from both hydrophobic and hydrophilic regions in these peptides,

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AMPs can interact with bacterial membranes to gain entry into cells. This characteristic enables them to attach to anionic (negatively charged) bacterial membranes and exert antibacterial activity. The interaction between AMPs and bacterial membranes primarily determines their antimicrobial function.[11,12] Amino acid diversity at the N-terminus of AMPs directly influences which AMPs can disrupt the membranes of specific bacteria while not being active against others.[13] The observations that cationic AMPs cause increased membrane disruption and permeabilization in bacterial membranes agree with this conclusion.[14]

The structural attributes of AMPs (including α-helices, β-sheets, combined α-helix and β-sheets, and extended structures; see Fig. 1)[15–18] give rise to distinct mechanisms of action. As the peptide interacts with the target membrane, it folds into an amphipathic α-helix.[19] The class of AMPs based on β-sheets is characterized by a strand of antiparallel peptide chains stabilized by two or more disulfide bonds.[15] It includes, for example, the defense peptides of vertebrates, insects, and plants.[20] There are high proportions of specific amino acids in extended peptides such as indolicidin, including tryptophan, histidine, and proline.[21] Most of these peptides adopt extended configurations upon interaction with the membrane. They are stabilized by hydrogen bonds and van der Waals forces with lipids rather than inter-residue hydrogen bonds. As the name suggests, loop peptides possess a loop structure imparted by the presence of a single bond, such as disulfide or amide.

Based on the structural features, the most important characteristic of all AMPs is their solubility in aqueous environments and their ability to partition into lipid environments.[22] As AMPs are generally cationic, they preferentially target bacterial membranes over zwitterionic eukaryotic membranes due to their anionic nature.[17,23–25] The hydrophobic amino acid content of AMPs facilitates the interaction of AMPs with cell membranes. AMPs that are non-ribosomal can also contain features such as lipids that may facilitate their interaction with

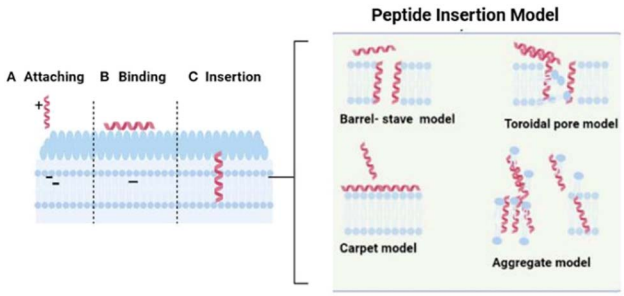

Fig. 2 The interaction between peptide and bacterial cellular membrane. This figure is based on the work of Saeed *et al.*[28]

membranes. As different organisms have different membrane compositions, AMPs have different selectivities.

Sometimes, AMPs inhibit biofilm production, cross the cell membrane, and inhibit cellular functions. However, AMPs generally cause cell death through membrane disruption and eventual cell lysis. Despite this, there are a variety of mechanisms of action among membranolytic AMPs have been proposed. A three-pronged mechanism of membrane disruption has been identified for ribosomally synthesized AMPs, particularly those that are helical or tetrahelical. "Barrel stave", "toroidal pore", and "carpet" are all terms describing these mechanisms.[18,23,26,27] (see Fig. 2).

AMPs of other types are less well-known regarding their mechanisms of action. In Fig. 3,[29–39] we summarize the most important AMPs and their relation to the source and mechanism of action.

As well as damaging the membranes, the peptide can kill bacteria by inhibiting the biosynthesis of nucleic acids, proteins, and some essential enzymes involved in synthesizing cell walls. The mechanisms for intracellular AMPs are summarized in Fig. 4.

The unique characteristic of the interactions involving AMPs is to inhibit a specific bacterium. It is believed that the molecular net charge of the peptide is the most important

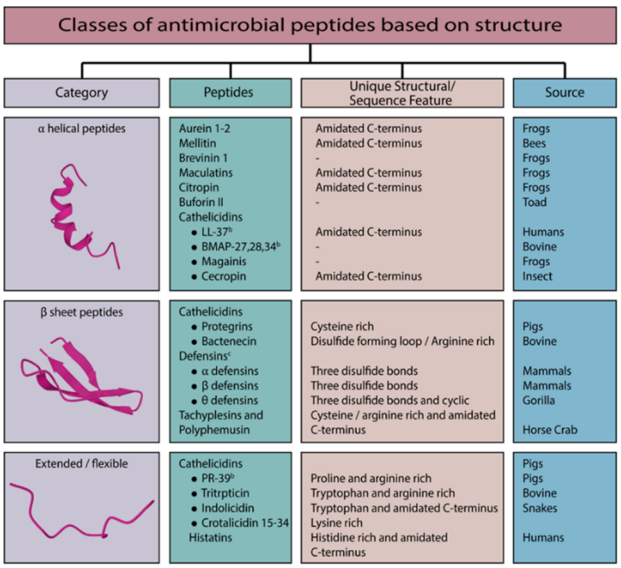

Fig. 1 Classes of antimicrobial peptides based on structure.

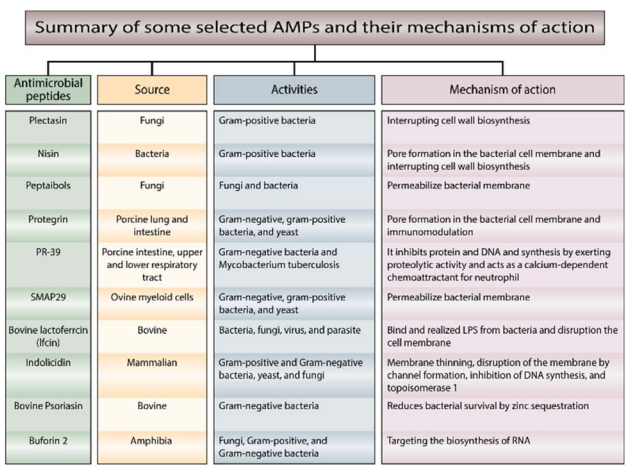

Fig. 3 Summary of selected AMPs and their mechanisms of action.

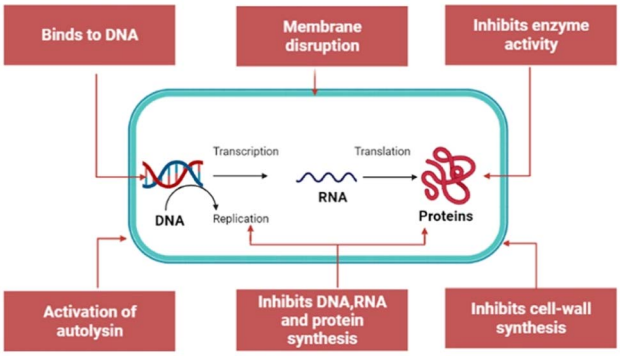

Fig. 4 Mechanism for intracellular AMP activity. This figure is based on the work of Saeed *et al.*[28]

characteristic that makes it effective against bacteria, as predicted based on AMP features contributing to antimicrobial activity.[40] Recent studies have also demonstrated that some other characteristics of AMPs play an important role in their antimicrobial activity, and these features may vary according to the bacterial species they target.[41,42] It is, therefore, possible to discover new characteristics of AMPs important for specific bacteria through a machine learning (ML) analysis of AMP characteristics associated with bacterium-specific efficacy. Doing so makes it possible to develop new antimicrobial drugs and better understand AMP's microscopic mechanisms.

AMPs constitute a promising class of compounds with a wide range of applications. This, in part, is a result of the diverse molecular chemistries of AMPs. However, herein lies the challenge of selecting and designing AMPs for novel applications. The number of potential AMP candidates for a specific application is enormous. Developing computational methods for selecting and designing AMPs is of utmost importance.

Computational strategies. To accelerate the design of antibiotic drugs, computational approaches can help interpret and guide the experiments. During conventional drug discovery and design, molecules are designed to target a particular protein in equilibrium through noncovalent interactions such as hydrogen bonding, ionic bonding, and hydrophobic interactions.[43,44] There are two general types of computer-aided drug design (CADD) approaches in existence: structure-based drug design (SBDD) and ligand-based drug design (LBDD). The SBDD method analyses the 3-dimensional structural information of macromolecular targets, typically proteins or RNA, to identify key sites and interactions essential to their biological activities. It can then be utilized to design antibiotic drugs competing with fundamental interactions involving the target to interrupt the biological pathways necessary for microorganism survival. An important aspect of LBDD is the identification of known antibiotic ligands for a target and the establishment of a relationship between the physiochemical properties of these ligands and their antibiotic activities, referred to as a structure–activity relationship (SAR). The computer-aided *de novo* drug design concept was first introduced more than 25 years ago.[45] It has been common practice to apply receptor- and ligand-based *de novo* design approaches when structural information is available. A method is applicable for determining target–drug interaction sites and receptor- and ligand-based scoring for selecting the most promising candidates.[46–48] AMP *de novo* design is a way to explore the number of new sequences. The junction with synthetic biology ideas represents a capable scenario for developing foldamers and biomimetic antimicrobial polymers that mimic AMPs for therapeutic purposes, for example.[49]

Faccone *et al.*[50] have demonstrated different strategies for generating effective drug candidates based on *de novo* algorithms. By combining computer-assisted approaches with omiganan (MBI-226) peptides, these authors have engineered an AMP. They identified functionally relevant natural or synthetic peptide motifs at specific amino acid positions. Database filtering technology (DFT) has also been proposed as a promising approach for the *de novo* design of improved AMPs. Mishra and Wang[51] first began exploring this concept. The features identified as filters for designing novel peptides were peptide activity, length, amino acid frequency, charge, hydrophobicity, and structure profile. It was shown that the obtained peptide efficiently inhibited a Methicillin-resistant *Staphylococcus aureus* (MRSA).

As a key tool for combating multidrug-resistant bacteria, quantitative structure–activity relationship (QSAR) methods provide useful information for the rational design of new active molecules at a minimal cost. Much progress has been made in QSAR methods, from traditional 2D-QSAR methods to 3D-QSAR methods, incorporating parameters such as molecular and spatial variety and protein flexibility. In QSAR, two of the most commonly used methods are comparative molecular field analysis (CoMFA) and comparative molecular similarity index analysis (CoMSIA), both of which are linear. QSAR methods have been widely used in discovering and growing different libraries for new antibacterial agents.[52–55]

Various linear and nonlinear statistical methods are used to develop these models based on the 2D or 3D representations of molecules. As a result of its simplicity, transparency, reproducibility, and ease of interpretation, multiple linear regression (MLR) is often used to obtain QSAR models. Due to the direct correlation between each descriptor's coefficient and its algebraic sign, it is easy to interpret its contribution to the model.

In pattern recognition, linear discriminant analysis (LDA) separates two or more class objects based on a linear combination of variables and can be applied to classification problems. The differences among data classes are explicitly modeled as part of the LDA method.

Nonlinear techniques (machine learning) are becoming more influential. Among the machine learning methods used in QSAR are artificial neural networks (ANNs), random forests (RFs), and support vector machines (SVMs).

In traditional QSAR models, the relationship between activities and the variables of the descriptors is identified. Additionally, RF (random forest) and DNN (deep neural network) methods from the machine learning approach were used to develop the prediction model. A decision tree (DT) is a classification method using ensemble learning. The final model was based on the highest score from individually developed trees in

the forest. The DNN algorithm is a mathematical method designed to mimic the neurons (nodes) of the human brain to recognize objects and analyze them progressively, improving previous neural network algorithms. Thus, more features are identified as more executed nodes are added to each layer.

Traditional *vs.* ML-based QSAR. The present perspective's primary focus is machine learning (ML)-based QSAR methods. It is, therefore, important to differentiate and emphasize the similarities between traditional and ML-based QSAR. As mentioned earlier, there are several important physical properties that an AMP candidate needs to satisfy. QSAR aims to predict physical properties with knowledge of AMP molecular structure. More formally, we wish to create a functional mapping where we map AMP molecular structure to a physical property of interest:

$$f : \boldsymbol{X} \mapsto y$$

where f is our function that maps from $\boldsymbol{X}$ (vector of descriptors) to y (scalar physical property of interest). The difference between traditional and ML-based QSAR is in the functional mapping, f. In both cases, we can use a similar set of descriptors, $\boldsymbol{X}$, and we will train on similar data to predict a desired physical property by minimizing the squared difference between the reference and predicted data. Using traditional QSAR, we are typically limited in our functional map and, as mentioned earlier, usually employ MLR, *e.g.*:

$$y = a_0 + a_1x_1 + a_2x_2 + \ldots$$

where x_i are the elements of $\boldsymbol{X}$ and a_i are model constants. On the other hand, with ML-based QSAR, the computer will "learn" and use a specified strategy (*i.e.*, ANN, RF, SVM, DNN) to develop an optimized functional mapping.

Using traditional models (*i.e.*, MLR), we end up with an analytic equation that can readily be presented and shared. On the other hand, with machine learning we do not have an analytical model that we can share and summarize, but instead some computer code that we can share.

Overview. In this perspective, we provide a brief overview of the previous and ongoing work on the latest scientific breakthroughs and technologies based on artificial intelligence (AI) and machine learning methods that could propose new options and alternative strategies for developing novel AMPs. However, cases exist where the applications of known AMPs have encountered development, production, and shelf-life issues. To drive the development of AMP-based treatments, it is necessary to create design approaches with higher precision and selectivity toward resistant targets, a generative approach to designing AMPs with experimental validation. The junction between these elements allows one to leverage and generate novel, diverse, and tailored candidates for specific applications, making it an efficient AMP design tool.

This Perspective article summarizes strategies for the design of the new AMPs. As part of our review, we addressed the challenges that *de novo* AI strategies for new AMPs must overcome. For example, proper molecular representation is a key point for *de novo* molecule generation. Model benchmark and how and which metrics should be used to evaluate the obtained models is the other Achille's heel of *de novo* design. A *de novo* molecule generation model benchmarking and validation can be challenging. To validate newly generated molecules, it is best to synthesize them and then test their predicted properties experimentally. This review aims to provide readers with the information and context needed to utilize generative modeling effectively.

Training database

The overarching goal of computational strategies for identifying and designing AMPs is two-fold. First, we seek methods to identify promising AMP candidates. Second, we strive to understand the underlying molecular-level properties of AMP candidates that lead to a specific activity. This second point is crucial for optimization and intuitive design applications—additionally, our computational strategies generally fall into two camps. First, molecular dynamic (MD) simulations (structural and mechanistic analyses) may be used to study AMP-target interactions in atomistic detail. Theoretically, this presents a way to identify promising AMP candidates and gain insight into the underlying intermolecular interactions. The MD simulations provide mechanistic insights into the different modes of action during the early stages of interaction and the time-dependent information about the structure of AMPs and the nature of the interactions. We have summarized the MD methods in Table 1. Simulating AMP-lipid interactions using MD simulations has been common practice for many years.[56–59] Almost all simulation studies, however, have been of AMPs that form stable α-helical or β-hairpin structures upon binding to and/or insertion into the membrane. These AMPs are generally more likely to act *via* a pore-forming mechanism. Less is known, however, about the mechanism of action of unstructured AMPs such as the linear battacin analogs. Unfortunately, such strategies are inefficient and impractical for early-stage design applications and the identification of candidates.

Second, machine learning-based quantitative structure–activity relation (ML-QSAR) methods can be used. The database used to train the model is central to successfully identifying AMP candidates. Within our earlier discussion, the role of the database is the development of the functional mapping from descriptor to property space. AMPs are short peptides containing up to 100 amino acids, with most AMPs containing less than 50 amino acids.[16,95] In the work of Sharma *et al.*, the authors considered AMPs contain 10 to 200 amino acids. If one were to try to enumerate all possible unique structures, the problem quickly becomes intractable.[96]

The database used to train the model must cover the important range of phase space, as the promising AMP candidates will be a subset of this. Put differently, we seek to interpolate within the dataset, if possible, compared to extrapolate. Data availability continues to expand, as does the computational ability to process massive amounts of information and readily available packages to train ML-QSAR methods. The dataset used to train ML-QSAR methods comprises AMPs (positives) and non-AMPs (negatives). In 2018, Bhadra *et al.*[97] constructed a positive database of naturally occurring and

Table 1 Summarized MD methods

Process/property of interest	Simulation technique	Considerations for AMPs
Peptide secondary structure	Conventional atomistic[60–66] Molecular dynamics (MD) Replica exchange approaches[67–71]	* Can be used to monitor conformational stability * Can capture slower peptide conformational changes than conventional MD * Fewer simulation repeats are needed, but it is still advisable to check that simulations starting from different conformations converge to the same point
	Accelerated (a) MD[72,73]	* Can capture slower peptide conformational changes than conventional MD
	High-temperature (HT) MD[69,74]	* Elevated temperatures can speed up the kinetics associated with peptide insertion and folding in the membrane
Peptide aggregation	Conventional atomistic MD[62,75–77]	* Can be used to monitor the stability of experimentally determined
	Coarse-grained (CG) MD[64,78,79]	* Larger systems and longer simulation times can be achieved * Atomic detail is lost
	Metadynamics[76,80,81]	* The technique can be used to enhance the sampling of slow aggregation event
	Umbrella sampling (US)[82,83]	* The technique can be used to enhance the sampling of slow aggregation
Peptide–membrane interactions	Conventional atomistic MD[62,84,85]	* Can be used to investigate the surface interactions of AMPs * Multiple simulation repeats may be required to achieve statistical significance
	CG MD[86–88]	* Larger systems and longer simulation times can be achieved atomic detail is lost
	US[85,89,90]	* US can be used with the reaction coordinate set as the center of mass (COM) distance between a peptide and the membrane
	Metadynamics[81,91]	* Metadynamics can be used to enhance the sampling
	HT-MD[74,92]	* Temperatures can speed up the kinetics involved in AMP insertion into the membrane and folding
	Electroporation[93,94]	* The technique forces the poration of bilayers and can, therefore, be used to increase the sampling of AMPs entering pores
	aMD[72,73]	* Increasing the sampling of peptide conformations should be able to escape energy minima quicker and access different metastable states

experimentally validated AMPs from APD3,[97] CAMPR3,[98] and LAMP[99] databases.

After eliminating duplicates and removing unnatural amino acids, they obtained a training database of 3268 AMPs. Their negative database was sourced from the UniProt database limited to proteins containing 5 to 255 amino acids.[100] After eliminating sequences labeled as AMP (and similar labels) and unnatural amino acids, they were left with 166 791 non-AMPs.

Subsequently, in 2021, Sharma *et al.* constructed a positive database using the protein database of NCBI (US National Center for Biotechnology Information)[101] and the StarPepDB database.[102–104] After eliminating duplicates, removing unnatural amino acids, and restricting the set to AMPs containing 10 to 200 amino acids, they obtained a training database of 10 187 AMPs. We note that by restricting the AMPs to 10 to 200 amino acids in length, only 576 AMPs were eliminated. The authors sourced their negative database from UniProt, again restricting the results to proteins containing 10 to 200 amino acids. After removing sequences labeled as AMP (and similar labels) and unnatural amino acids, they were left with 10 422 non-AMPs.

The works of Bhadra *et al.* and Sharma *et al.* highlight important issues concerning the database used to train. First, they found that there is no universal AMP database. Moreover, overlaps between the databases exist. In Table 2 below, we summarize the most important general AMP databases. Table 2 follows Ramazi *et al.*'s recent review, providing additional details on each database.[105]

Additionally, care needs to be taken concerning the data coverage; the ML-QSAR-identified candidates depend on the database's quality.[122] Sharma *et al.* only considered AMPs

Table 2 Summary of the available public AMP databases

Database name	Number of covered classes and AMPs	Size	Type of database	Type of data	Years	URL
dbAMP 3.0	52 biological activities in 3044 organisms	~57 304	Exp. and pred.	Natural and synthetic	2023	http://awi.cuhk.edu.cn/dbAMP
dbAMP 2.0 (ref. 106)	52 biological activities in 3044 organisms	~28 709	Exp. and pred. secondary	Natural and synthetic	2021	http://awi.cuhk.edu.cn/dbAMP
dbAMP[107]	26 biological activities in 2048 organisms	~12 389	Exp. and pred. secondary	Natural and synthetic	2018	http://csb.cse.yzu.edu.tw/dAMP/
DBAASP[108]	Antibacterial, antifungal, antiviral, anticancer, and antitumor in seven organisms and cancer cells and mammalian cells	~15 700	Exp. and pred. secondary	Natural, synthetic, and patent	2021	http://dbaasp.org/home
LAMP[109]	8 major functional classes and 38 functional activities	~23 250	Exp. and pred. secondary	Natural, synthetic, and patent	2020	http://biotechlab.fudan.edu.cn/database/lamp/index.php
DRAMP[110]	Antibacterial, antifungal, antiviral, anticancer, antitumor, antiprotozoal, and insecticidal	~22 250	Exp. and pred. secondary	Natural, synthetic, patent, and AMPs in drug development	2019	http://dramp.cpu-bioinfor.org/
InverPep[111]	Invertebrates phyla Arthropoda, Mollusca, Nematoda, Annelida, Echinodermata, Platyheminthes, Placozoa, the Hydridae family (Cnidaria) and the subphylum Tunicata (Chordate)	~770	Exp. primary	Natural	2017	http://ciencias.medellin.unal.edu.co/prospeccionydisenobiomoleculas/InverPep/public/home_en
CAMPR3 (ref. 112)	Antibacterial, antifungal and/or antiviral	~8160 sequences and 757 structures	Exp. and pred.	Natural, Predicted and patented	2016	http://www.camp3.bicnirrh.res.in/
MEGARes[113]	Antimicrobial compounds, *e.g.*, drugs, biocides, multi-compound, and metals	~9000	Exp. primary	Natural	2022	http://megares.meglab.org/
ADAM[114]	Archaea, bacteria, plants, and animals	~7000	Exp. primary	Natural	2015	http://bioinformatics.cs.ntou.edu.tw/adam/index.html
APD[115]	Antibacterial	~1230	Exp. and pred. primary	Natural and patent	2008	https://webs.iiitd.edu.in/raghava/satpdb/catalogs/apd2/
Defensins knowledge-base[115]	Defensin, antimicrobial	~360	Exp. primary	Natural	2007	http://defensins.bii.a-star.edu.sg/
YADAMP[116]	Antibacterial	~2525	Exp. and predicted	Natural	2018	http://www.yadamp.unisa.it
MLAMP (unbalanced dataset)[117]	Antibacterial, anticancer, antifungal, anti-HIV and antiviral	~879 AMP ~2405 non-AMP	Predicted	Predicted	2016	http://www.jci-bioinfo.cn/MLAMP
DADP[118]	Antimicrobial, antibacterial, anticancer	~2571	Prediction	Prediction	2012	http://split4.pmfst.hr/dadp/
Bactibase[119]	Bacteriocin	~230	Prediction	Predicted	2010	http://bactibase.hammamilab.org/main.php
BAGEL4 (ref. 120)	Bacteriocin	~814	Prediction	Predicted	2018	http://bagel4.molgenrug.nl/index.php
ADAPTABLE[121]	Antimicrobial, antibacterial, antifungal, anticancer	>40 000	Prediction	Predicted	2019	http://gec.u-picardie.fr/adaptable

containing 10 to 200 amino acids. While only a small number of AMPs were eliminated, AMPs are generally short in length to prevent issues related to folding in large AMPs.[123] Both Bhadra *et al.* and Sharma *et al.* also eliminated unnatural amino acids. While this is a common practice, doing so also removes the possibility of the ML-QSAR method to identify AMP candidates containing unnatural amino acids. However, the recent work of Murakami *et al.* demonstrates that including unnatural amino acids in the design of anti-bacterial AMPs may be advantageous.[124]

One needs to be careful concerning the source of the data. From Table 2, we consider the most recent available version (2.0) of dbAMP available online.[125] The database currently contains 28 709 AMPs. Of these, 18 345 (63.9%) are validated, and the remaining 10 364 (36.1%) are predicted. Fortunately, in dbAMP, the predicted AMPs are labeled and can readily be filtered out. This is indicated in Table 2 in that the primary database contains experimentally validated AMPs, with a secondary database containing predicted AMPs. This split is an improvement over earlier releases of the database. As posted on the previous dbAMP website, in the dbAMP 2.0 database released on 30 June 2021, only 31.6% (9062 of 28 709) of the AMPs were validated. Similarly, in the dbAMP database released on 15 June 2018, only 34.5% (4271 of 12 389) of the AMPs were validated.

Further, using the available online filters within the 18 345 validated AMPs contained in the most recent available version (2.0) of dbAMP available online, we find that the majority (11 431; 62.3%) contain fewer than 20 amino acids, 16 599 (90.5%) contain fewer than 40 amino acids, and 17 495 (95.4%) contain fewer than 60 amino acids, further emphasizing that AMPs are commonly short peptides. Furthermore, within dbAMP, it is noted that 2262 of the compounds are considered antimicrobial proteins, which are longer.[126]

The diversity of the database is also of the utmost importance. It has previously been found that many of the common databases are unbalanced concerning AMP activity, which presents challenges for ML-QSAR and classification methods.[127] Within the most recent available version (2.0) of dbAMP available online, let us consider the biological function of the AMP. Of the 18 345 validated AMPs, 13 538 (73.8%) are classified as anti-bacterial, the largest group within the database. For comparison, only 1592 (8.7%) validated AMPs are classified as anti-viral. The major ratio of the predicted AMPs within dbAMP (58.7%) is anti-bacterial. We note that the anti-bacterial class is further broken down into eight types.

The mode of action and the AMP molecular structure are dependent on the biological function of the AMP. For example, anti-bacterial AMPs generally contain hydrophobic cationic amino acids, which interact *via* electrostatic interactions with the negatively charged bacteria surface, leading to membrane disorder.[128] Conversely, anti-viral AMPs may bind to the target (DNA or RNA) to prevent virus replication.[129,130] Having found that most of the verified AMPs in the dbAMP database are anti-bacterial, ensuring the employed database is suitable for the desired application must be taken. In the same vein, the recent work of Murakami *et al.* demonstrates that the inclusion of unnatural amino acids, which are likely not well represented in the database, can affect the α-helical structure of the AMP and increase its anti-bacterial activity while reducing the net charge.[124]

In addition to the need for an AMP (positive) database, it is also essential to have a non-AMP (negative) database, which is a database of peptides validated not to exhibit antimicrobial behavior. In comparing the work of Bhadra *et al.* and Sharma *et al.*, the size of the negative database sourced from UniProt is relatively unchanged. On the other hand, the positive AMP database's size change is significant. Moreover, a major effect of this is that the positive to the negative ratio (P: N) is close to 1 : 1. When developing classification methods, the conventional goal is to obtain a P : N ratio as close to 1 : 1 as possible. In the work of Bhadra *et al.*, their P : N ratio was not close to 1 : 1. Interestingly, using their positive database and varying their negative database, they investigated the sensitivity of their model on the P : N ratio used. The authors suggest that a P : N ratio of 1 : 3 was best, in line with the databases they used. However, further studies are needed. The conventional practice of a P : N close to 1 : 1 is analogous to the desire for an AMP database equally distributed with respect to activity.

Care must be taken when assembling reference AMP and non-AMP data to train an ML-QSAR or classification method. Further studies are needed to understand the effect of the dataset on the resulting predictions. As raised by Elliott *et al.*,[123] we also question whether conventional design schemes and rules for small molecules apply to the design of AMPs. We imagine, for example, Lapinski's Rule of 5.[131] Could we learn from our conventional strategies and leverage them (*i.e.*, as filters) in assembling a database to train our models?

The database is crucial for developing the functional map from the descriptor to the physical property space. Next, we will discuss suitable descriptors required to develop the desired quantitative structure–activity (or property) relationship (QSAR) to make the most of the AMP and non-AMP databases. In chemical and materials informatics, descriptor and fingerprint are terms used interchangeably to describe heuristically determined molecular properties that are easier to calculate than the quantities one wishes to predict. During the development of quantitative structure–property (or activity) relationship (QSPR or QSAR) techniques, one uses the database (reference chemical property space) with the descriptors along with a suitable cheminformatics approach (functional map[132]) to make predictions (explore the desired chemical property space).

Molecular features

Generally, the descriptors used to model peptides are the same as those used in conventional small molecule drug design.[133] The detailed representation of larger molecules, such as peptides or polymers, might be more closely related to features' overall distribution and spatial arrangement. Therefore, the same methodology used to model small molecules may not be used directly. In this regard, it was proposed that the representations of peptides be simplified into amino acid (AA) scales,

where each AA side chain is assigned a value for its characteristic value representing the whole molecule.[134,135]

There are different ways in which peptide sequences can be processed based on the AA scales. In either case, a global average value is calculated for all side chains in the sequence or the values are computed based on the position of the corresponding AA in the sequence. There are several ways to retain such positional information.[136] The most widely used method involves autocorrelation and cross-correlation measures on discrete descriptor scales. To serve this need, extensive research has been devoted to developing freely available and commercial packages of molecular and quantum mechanical-based descriptors, with several excellent reviews and comparisons available.[137–140] The descriptor packages can be facilitated using freely available online servers.[141–143] Nonetheless, their application is not without challenge. The issue is twofold. First, it is desirable to not only use QSPR (or QSAR) to predict suitable AMPs for a particular application, but it is of great value to gain insight into the underlying molecular-level driving forces for intuitive (early stage) design processes. Too often, insight is clouded by the employed descriptors. Consider that the commonly used and freely available PADEL package contains 1875 descriptors, while the popular commercial DRAGON package contains 4885 descriptors. This leads to the second challenge of overfitting.[138]

Within QSPR (or QSAR), the primary technique to reduce the number of descriptors by identifying interrelated descriptors is by a principal component analysis (PCA).[144] This reduces the number of parameters to prevent overfitting and can help highlight the essential molecular features. Related to this, Bhadra *et al.*[97] developed an ML-based AMP classification method. Using the Distribution (DF) descriptor set from the Global Protein Sequence Descriptors, they could reduce the number of descriptors used from 80 to 23 while maintaining high accuracy.[145] Kleandrova *et al.*[142] have developed a multitask computational model utilizing Moreau–Broto autocorrelation descriptors to predict the activity and cytotoxicity of AMPs. ModlAMP is a software package that includes functions for calculating correlated descriptors for various AA scales. Furthermore, peptide descriptors can be classified according to different AA scales: one-dimensional or global descriptors, which average over the whole sequence, and multi-dimensional descriptors, partly keeping positional information.

Future efforts on the development of descriptors for use with AMPs will be of great value. Existing property–activity relationships can be leveraged, such as AMPs with anti-bacterial activity commonly have a significant net positive charge. Moreover, one should consider key structural features that distinguish peptides from small molecules, such as their large size and flexibility and their make-up from a series of amino acids. If descriptor sets can be constructed containing only the most important features of AMPs, it can help provide insight into the underlying structural property relationships. The molecular structures can be used to calculate all the previously mentioned representations.

In addition to activity, AMPs must exhibit various properties to be effective for a specific application, which can be used as descriptors. A fundamental property of interest is the octanol/water partition coefficient, log *P*, measured as the equilibrium distribution of a dilute peptide (solute) between water and octanol-saturated phases. The octanol/water partition coefficient is commonly used to characterize the lipophilic/hydrophilic balance of the peptide. It is an important parameter to determine the fate of the peptide in the body for pharmaceutical applications. Leo *et al.*[143] provide a comprehensive review of partition coefficient theory.

Nonetheless, log *P* is limited in only considering the partitioning of a single peptide form. To overcome this limitation, the octanol–water distribution coefficient, log *D*, considers protonation, deprotonation, and tautomerization.[146] The task of measuring log *P* and log *D* for small molecules can be difficult, cumbersome, and imprecise, which we expect to be even worse for the case of peptides.[147]

Given the difficulty in measuring log *P* and log *D*, there is an excellent opportunity to use structural-based descriptors and computational methods to make predictions. We see three reasons for this. First, as already described, log *P* and log *D* are great physical values. Second, they can provide insight into the important AMP characteristics for a particular activity. Log *P* and log *D* may offer insight during classification processes and ML-QSAR methods to identify and design AMPs for a specific application. Moreover, log *P* and log *D* may be used as descriptors themselves. Third, previous work has demonstrated that conventional QSAR tools trained on small molecules are not suitable for predicting log *P* of peptides.[148] This is not unexpected, given the complex chemistry of peptides. This, therefore, presents an opportunity for transfer learning, wherein one could first develop an ML-QSPR method to predict the log *P* of AMPs. This would allow one to identify the most important peptide descriptors, which could be used to predict additional properties and activities.

Similarly, in the work of Zhou *et al.*,[147] HPLC retention times have been used to study amphipathic helical peptides. They found that the retention time of the peptides correlates with their antibacterial properties, as demonstrated in the case of amphipathic helices, which have been found to have antibacterial properties. Using a reverse approach, Meek *et al.* were able to predict retention times for peptides up to 20 amino acids in length.[148] As measured by CD spectroscopy, other empirical properties used as peptide descriptors are aqueous solubility, refractive index, and helicity. Strøm *et al.* performed a multivariate analysis of several empirical properties for modeling variants of murine amplactoferricin.[149] According to the authors, helicity and global charge were the most critical factors determining the activity of peptides.

Leveraging AI for expanding the AMP space

There is an urgent need for computational methods that promise to expedite the discovery of new drugs because of the proliferation of drug-resistant pathogens and the slow and costly development of antibiotics. In this part of the review, we

describe advances in discovering AMPs (antimicrobial peptides) facilitated by AI (artificial intelligence). Given the antimicrobial resistance crisis, we analyze best practices in AI-driven antibiotic discovery and advocate for openness and reproducibility to accelerate preclinical research. As a final point, the literature trends and areas for future research are discussed, as AI enhancements to drug discovery at large provide many opportunities for future applications in antibiotic development.

Available improvements in ML and deep learning technologies, particularly deep learning techniques, have demonstrated their favorable impact on generative chemistry and computer-aided drug discovery.[150,151] The application of ML to drug discovery, and antibiotic discovery specifically, has been greatly facilitated by the public availability of empirical datasets (Table 2), advances in computer engineering, and the proliferation of free and open-source ML libraries.

Several deep learning techniques, including generative adversarial networks (GANs),[152–154] have been used to develop novel peptides and proteins for drug targets in generative chemistry during the drug screening and discovery stages of the drug development process. In light of the above, it has been indicated that deep learning techniques such as GAN algorithms will be essential to the future of generative chemistry as well as computer-aided drug discovery and design, as these advantageous approaches can be applied to numerous aspects of generative chemistry and drug discovery by computer. Several drawbacks of the ML can be attributed not only to the selection of an appropriate model and/or use parameters, but the question is how to scale the selected descriptor features and handle unbalanced data classes.[155] We are dealing with a data-rich library based on a sequence representation for the peptides (and/or small molecules), which can be represented by a set of features (numbers) describing the molecule's characteristics in a machine-understandable way. The question of which representation to choose or drop for a given problem is feature selection.[156] There is a problem with this inequality in ML models, which must be eliminated to prevent overestimating single features by their magnitude rather than their real underlying issue. However, there was a lack of consistency and reproducibility in the application and robustness of AI-based antibiotic discovery models. It will be necessary for antibiotic discovery to be accelerated using computer-aided approaches for new drugs with novel mechanisms of action (MOAs[157]), for example, with the application of ML techniques such as support vector machines (SVMs[40,158]).

A wide range of chemical space is available to design computational antibiotics. In recent years, the developments in the gut microbiome and progress in sequencing analysis opened a new avenue for harvesting antibiotic-resistant genes, and the human gut is an alternative reservoir for AMP structures.[159] According to bioinformatics analysis, many potential AMP families in the human gut microbiome remain to be studied. The large number of potential AMPs derived from the human gut microbiome, thus, theoretically, could serve as a source of candidates against infectious bacteria.[160]

Using artificial intelligence approaches, such as natural language processing (NLP), it is possible to identify candidate AMPs by identifying sequence features from genome sequences, even short sequences with low homology, and features from DNA sequences. Ma *et al.*[161] demonstrate that combining neural network models (NNMs) for autonomous learning of AMP sequence features and large-scale human microbiome data resources can discover AMPs with high antibacterial potency.

A closed-loop approach combining experimental and machine learning techniques requires a template with known antimicrobial activity and a series of homologous sequences. Using a generalized linear model, new AMPs with 160-fold higher antimicrobial activity against *Escherichia coli* could be created by training a generalized linear model.[162] Most machine learning-based antibiotic development approaches utilize molecular descriptors space exploring as the basis of new representations for drug candidates and new models to predict their activity. In contrast, phenotypic drug discovery emphasizes the molecule's effects on target organisms rather than the molecule itself. Using cell imaging, for example, a recent study used a random forest model to predict antimicrobial activity without describing each molecule individually.[163] A focus on the effects of drugs on pathogens, rather than comparing molecular descriptors directly, can expand the search space for new medicines.

In recent years, deep learning techniques have made it possible to model generative adversarial networks (GANs) that can be used to design new peptides and proteins. As opposed to artificial neural networks consisting of only one layer, deep learning uses artificial neural networks that consist of multiple layers.[164]

Generative modeling reframes molecule design as an inverse design problem, which provides an alternative method of discovering new molecules.[165] Generative models offer a promising solution. By leveraging recent advances in deep learning, generative models help to solve the inverse molecular design problem: what set of molecules will satisfy a given set of properties? Generic models enable rapid identification of diverse sets of molecules highly optimized for specific applications by identifying a function that maps properties to structures.

Deep neural networks are highly dependent on their architecture, which consists of the types of layers and how they are arranged. The classification of deep generative models for molecular discovery can be divided into three classes: variational autoencoders (VAEs), generative adversarial networks (GANs), and normalizing flow models. A VAE is a generative model consisting of an encoder, which maps molecules into continuous embeddings, followed by a decoder, which reconstructs molecules based on the learned embeddings.[166] VAEs are directed probabilistic models, learning continuous latent variables through a variational Bayesian approach to generative DL.

The loss function of VAEs consists of two terms: (1) a reconstruction loss which forces the decoder to recover the correct molecule from the embedded structure, and (2) a Kullback–Leibler divergence term that regularizes the distribution of learned molecular embeddings so that the distribution of generated molecules closely resembles the distribution of

training molecules. In molecule generation, VAEs have been used to generate SMILES strings and molecular graphs.[167–170]

As a first step, it is necessary to formulate these distinct applications as concrete problem statements; for example, we seek to discover molecules with X properties subject to Y constraints. Broadly, molecular generation problem statements fall into three classes: (1) unconstrained molecular generation, (2) property-constrained molecular generation, and (3) structure-constrained molecular generation.

By appropriately tuning models and their associated latent spaces, targeted sampling of new antimicrobial peptides with ideal characteristics can be achieved. For the relevant test case of generating novel antimicrobial peptide sequences, Renaud and Mansbach[170] focus on the question of the quality of -latent spaces and their interpretability. To evaluate and compare the different behaviors of deep generative models with VAE-like latent spaces in terms of reconstruction accuracy, generative capability, and interpretability, we will use deep generative models with VAE-like latent spaces. In specific regions of the latent space, the obtained models can generate unique and diverse sequences and grow more AMP-like.

An overview of deep generative models for peptides was presented in a recent review.[171] Several challenges still need to be addressed. For example, no single deep generative model framework consistently produces superior results compared to other deep generative models. Due to this, selecting an appropriate model from various deep generative frameworks can be challenging given a peptide dataset of interest. Additionally, benchmarking datasets and metrics in peptide generation evaluation are lacking, further complicating comparing and selecting models. There have been several benchmarking platforms developed in the field of molecular generation, including GuacaMol[172] and MOSES,[173] that use a variety of criteria to assess the quality of the generated data, such as novelty, uniqueness, validity, and Fréchet ChemNet distance. A similar benchmarking platform for peptide generation models is urgently needed.

Since generative modeling criteria may vary from application to application, developing a set of benchmarks is difficult. In an ideal benchmarking set, metrics relevant to a wide range of applications would be included, and solutions to most of the obstacles associated with using generative models for molecular discovery. We anticipate this set of benchmarks to include synthetic feasibility, safety and handling, uncertainty quantification, and other relevant factors relevant to deploying generative models in real-world applications.

GAN-based algorithms can go beyond other models only when the generative network module can generate continuous output values, such as a vector of numbers, as in the image generation task. Using a vector of numbers, we can train the generative network module and adjust its weights based on the gradient of the loss function from the discriminative network module. Nevertheless, peptide/protein structures are represented in text strings, not continuous numbers. This is one fundamental trick of GAN-based algorithms in *de novo* peptide and protein design. Thus, we must design an approach to facilitate gradients through peptide/protein structures. The existing solutions in the literature are as follows. First, a pairwise distance matrix between α-carbons on the protein backbone represents protein structures. Second, a four-dimensional (4D) tensor is employed to describe the positions of active atoms in proteins. Third, DNA/gene sequences are used and converted into protein sequences. Moreover, Ramachandran angles, the main chain torsion angles (*i.e.*, phi and psi) in each amino acid, are used to represent a protein structure. In addition, the generative and discriminative network modules directly deal with a latent vector encoded by 20 canonical amino acids.

For the deep generative models, we should consider the limitations arising from the dynamic and conformational states of the peptides. Input PDB structures of peptides may not contain sufficient information for computational modeling, for example, due to the static condition. It won't be possible to capture enough data for computational modeling. An alternative to using a single PDB structure for the generative models is to take a set of peptide conformers or a trajectory of structure changes (computed using molecular dynamics) as inputs. We anticipate that deep generative models will play a significant role in drug discovery in the future as we become more adept at generating structural and functional data about peptides and deep learning advances. Then, in our opinion, coupling algorithms should be proposed and developed in the future to overcome these problems with the flexibility of the AMPs, but which is the main property in the other way. Degiacomi[174] presents a usage of generative neural networks for the characterization of the conformational space of proteins featuring domain-level dynamics. The generated protein-like structures can be sampled with a protein–protein docking algorithm to score the conformations (poses) close to the bound state. In addition to pre-existing MD simulation data, the autoencoders can generate new, realistic AMP conformation space. Suppose there is a sufficiently large dataset available. In that case, it may be possible to train a general neural network suitable for molecular modeling that can be rapidly trained using transfer learning to tackle a specific conformational space sampling problem.

Outlook and future perspective

Antimicrobial peptides hold great promise in addressing a wide range of pressing issues. They are natural-based compounds with unique chemistries. As the availability of reference data continues to grow, computational methods are poised to make significant contributions to the selection and development of antimicrobial peptides for specific applications. This is aided by the increased availability of computational tools (machine learning and deep learning) and readily available, user-friendly software. As the field advances, we must establish best practices. We also believe that rather than thinking of antimicrobial peptides in a vacuum, we must recall what has been successfully done in small molecule drug discovery and leverage their known physical properties.

It is anticipated that the current challenges in generative modeling will be overcome in the coming years, even though many advances are still required for generative modeling to achieve its full potential.

Data availability

As this is a Perspective article, no primary research results, data, software or code have been included.

Author contributions

Conceptualization: M. N. and M. L.; investigation: M. N., A. S. P., D. P. V., M. L.; writing – original draft: M. N., A. S. P., D. P. V., M. L.; writing – review & editing: M. N., A. S. P., M. L.; visualization: M. N.; project administration: M. N.

Conflicts of interest

There are no conflicts to declare.

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