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Animal Diseases

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Functional immunopeptides: advancing prevention and therapeutic strategies against animal diseases

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Abstract

Peptide-based therapies have emerged as groundbreaking advancements in both therapeutic and preventive strategies against infectious diseases. These approaches utilize innovative functional immunopeptides—such as antigenic peptides, antimicrobial, immune modulation, and delivery peptides derived from pathogens or hosts—to target specific immune mechanisms. In addition to their simplicity of use, peptide-based approaches provide several advantages. These include improved specificity and immunogenicity by targeting specific antigenic peptides and enhanced delivery of particular proteins or vaccines to targeted immune cells, which increases the efficiency of antigen presentation and provides a self-adjuvant effect and therapeutic properties. The most recent developments in peptide-based systems to increase vaccine efficacy and therapeutic interventions for animal diseases are investigated in this review. It encompasses fundamental ideas, immunomodulating functions, and peptide production techniques. Additionally, the improvements and synergistic advantages attained by combining these functional immunopeptides with vaccines or using them as stand-alone therapeutic agents are emphasized. This review demonstrates how peptide-based treatments in veterinary medicine enhance immune responses and inhibit or eliminate pathogens.

Keywords Immune regulation, Peptide-based strategies, Vaccine optimization

Introduction

Recent advancements in vaccination technology have enabled novel approaches, such as nanoparticles and functional immunopeptides. Composed of short amino acid sequences, these functional immunopeptides enhance vaccine efficacy and act as therapeutic agents against pathogens. Antigenic peptides, antimicrobial peptides, immune modulation peptides, and delivery peptides are among the several varieties they fall into (Fig. 1). The distinct functions and features of several types of functional immunopeptides are displayed in Table 1. By focusing the immune response on specific epitopes, optimizing antigen delivery, targeting particular immune cell receptors, and fostering immunostimulant effects, this approach seeks to inhibit particular pathogens and improve vaccination efficacy (Hamley 2022; Malonis et al. 2020).

Antigenic peptides are short protein segments that can bind to antibodies, stimulate an immune response, and be identified by immune cells, especially B cells and T cells (Lund et al. 2013; Parker et al. 1995). Antigenic peptides play an important role in vaccine enhancement

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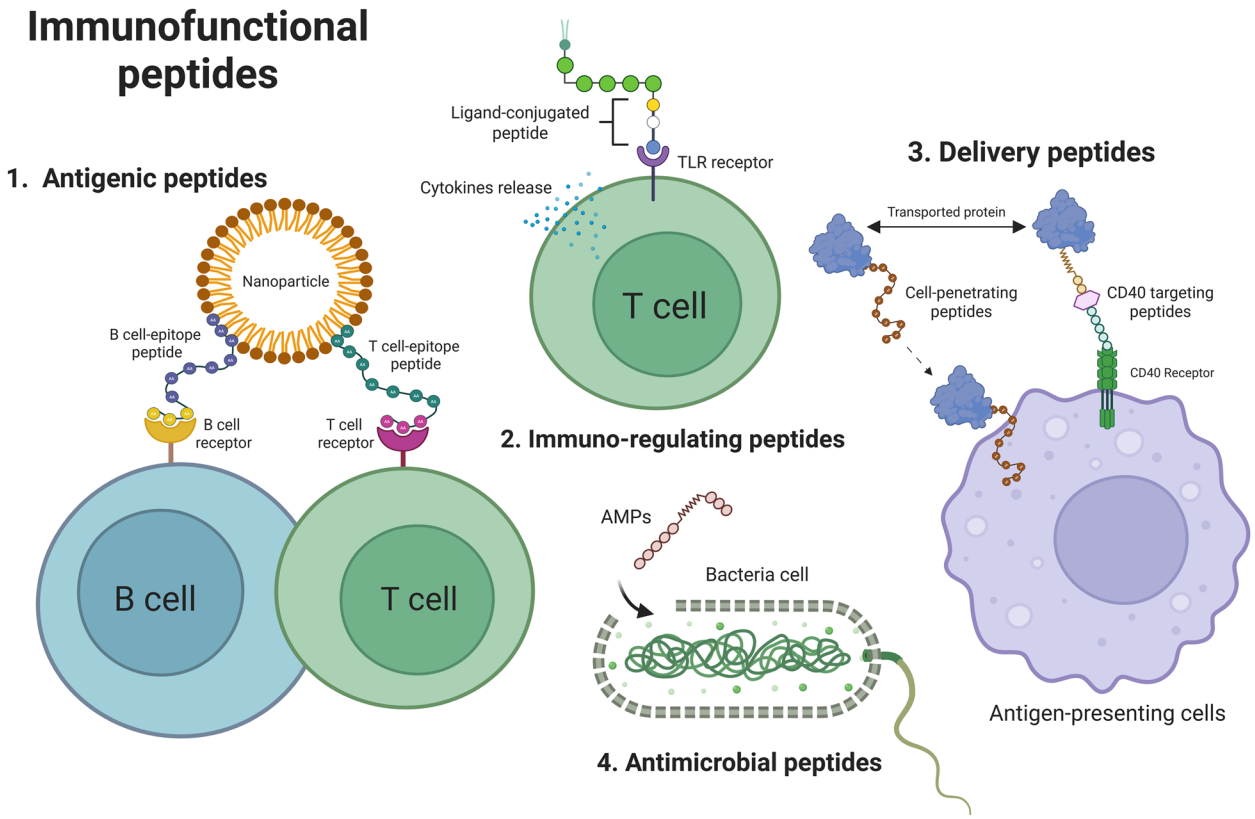

Fig. 1 Innovative functional immuno-peptides, including antigenic peptides, immune modulation peptides, antimicrobial peptides, and delivery peptides

Table 1 Functional immuno-peptides comparison

Characteristic	Antigenic epitopes	Immune modulation peptides	Antimicrobial peptides	Delivery peptides
Primary function	Induce specific immune response	Immunostimulant and adjuvant-like effects	Inhibiting or killing microorganisms	Enhance antigen or biomolecule delivery into cells
Target structure	Linear or conformational	Ligand-conjugated variable	Amphipathic α-helical or β-sheet	Variable
Specificity	Highly specific to the target pathogens	Highly specific to receptor	Broad-spectrum activity	Highly specific to receptors
Mechanism of action	Recognized by immune cells	Receptor binding	Variable	Receptor binding or cross-membrane
Stability	Generally stable	Generally stable	Often short half-life	Generally stable
Immunogenicity	High (by design)	Moderate to high	Variable	Low to moderate
Application in vaccines	Direct target (vaccine design)	Vaccine adjuvant	Potential antimicrobial vaccines	Enhance vaccine efficacy and the potential as the delivery vehicle for vaccines
Customization source	Epitopes from pathogens	Ligands to PRRs	Ligands to microbial functional targets	Ligands to APC or cargo receptors
Delivery efficiency	-	Variable (depend on design)	Variable (depend on formulation)	Improve antigen delivery and penetration to APC
Toxicity concerns	Low	Low	Potential toxicity to host cells	Low

Abbreviations: *PRR* pattern recognition receptor, *APC* antigen-presenting cell

by acting as immunogenic stimulants that improve the immune response to specific antigens. These peptides vary in length and may incorporate epitopes capable of activating B cells, T cells, or both. Their versatility in design and ability to showcase multiple epitopes together help boost immune recognition and immunogenicity (Apte et al. 2016; Chen et al. 2020; Joshi et al. 2013; Zeigler et al. 2019).

Small peptides, known as antimicrobial peptides (AMPs), are key components of the innate immune system and offer wide-ranging protection against various microorganisms. AMPs, which are generally composed of 10 to 60 amino acids, demonstrate antimicrobial activity as effectively as conventional antibiotics do. The potential of these compounds to help reduce bacterial drug resistance makes them promising options for developing new peptide-based therapies in the future (Huan et al. 2020; Lei et al. 2019; X. Ma et al. 2024; Talapko et al. 2022). AMPs play a significant role in immune response modulation. They act as a link between the innate and adaptive immune systems by activating immune cells and promoting cytokine production and chemotaxis (Duarte-Mata & Salinas-Carmona 2023; Ganz 2003; Q.-Y. Zhang et al. 2021a, b, c).

Immune modulation peptides are specialized peptides that help activate specific immune responses. They are divided into ligand-conjugated peptides and adjuvant-like peptides. Ligand-conjugated peptides are utilized primarily to bind to specific pattern recognition receptors (PRRs), i.e., NOD-like receptors (NLRs), Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and stimulators of interferon genes (STINGs) (Hamley 2022; T. Zhao et al. 2023). PRRs play a central role in the maturation of innate immune cells such as dendritic cells and macrophages. The maturation and activation of innate immune cells trigger the release of chemokines and proinflammatory cytokines, which, in turn, stimulate other immune cells, such as T cells and B cells, ultimately fostering adjuvant effects. Adjuvant-like peptides can function as immunostimulants without being conjugated to specific ligands. Like traditional adjuvants, they directly stimulate TLRs and boost immune responses through multiple mechanisms, such as creating an antigen depot, activating innate immunity, and costimulating immune cells (Azmi et al. 2014).

Delivery peptides are short sequences of amino acids that can be attached to a specific site or receptor on immune cell surfaces to promote antigen or transported protein uptake. This feature allows the delivery of peptides to deliver a drug, protein, or vaccine to the target cell by binding to specific APC membrane receptors (targeting peptides) or directly penetrating the antigen-presenting cell (APC) membranes (cell-penetrating peptides) (Melgoza-González et al. 2023; Todaro et al. 2023). In addition, targeting peptides can also inhibit pathogen attachment and entry sites by binding to specific receptors (Deng et al. 2023). Peptides that target specific receptors or surface markers in immune cells have been developed and may bind to specific receptors, including those that target CD45 and CD8 (T cells), CD11b and CD163 (dendritic cells and macrophages), CD177 and GR-1 (neutrophils), CD16 and NK1.1 (NK cells), and others (Todaro et al. 2023; H. Yang et al. 2024). By targeting specific dendritic cell (DC) subsets or direct delivery through membrane penetration, Major histocompatibility complex (MHC) class I and II presentation is enhanced, antigen uptake is improved, and a strong CD8+ and CD4+ T-cell response is elicited.

Vaccines incorporating ligand-conjugated or adjuvant-like peptides exhibit self-adjuvanting properties and enhanced immunogenicity. This leads to increased immunogenicity while also allowing for a dose-sparing effect and increased efficiency (Luchner et al. 2021; T. Zhao et al. 2023). Furthermore, the impact of ligand-conjugated peptides and targeting peptides on immune responses and vaccine immunogenicity is specific to the targeted cell and the types of receptors involved (Cifuentes-Rius et al. 2021; Matsuda et al. 2022). Recent innovative peptide strategies have shown potential in vaccine design and enhancement, particularly in the field of veterinary medicine. The focus of this review is to provide an in-depth understanding of recent peptide research and development in combination with vaccines and immunotherapies against animal infectious diseases. This review covers the characterization, generation methods, effects, stimulation of the immune response, and current status of implementation of this research.

Antigenic peptides

Antigenic peptides derived from pathogen-associated antigens function as critical immune response inducers through two primary mechanisms: MHC binding and epitope presentation. These peptides can induce a strong immune response by delivering antigenic sequences to immune cells or effectively binding to MHC molecules. This binding facilitates recognition by T cells, leading to the activation of both CD8+ and CD4+ T cells, thus promoting optimal immunogenicity (Malonis et al. 2020; Stephens et al. 2021). The most common antigenic peptide used in vaccine development for various animal diseases is an epitope-based peptide (Calis et al. 2013; W. Li et al. 2014; TopuzoĞullari et al. 2020).

Epitope-based vaccines are categorized by epitope type, including T-cell (CD8+/CD4+), linear B-cell, multiepitope, and conformational epitopes (Parvizpour et al. 2020). When developing subunit vaccines,

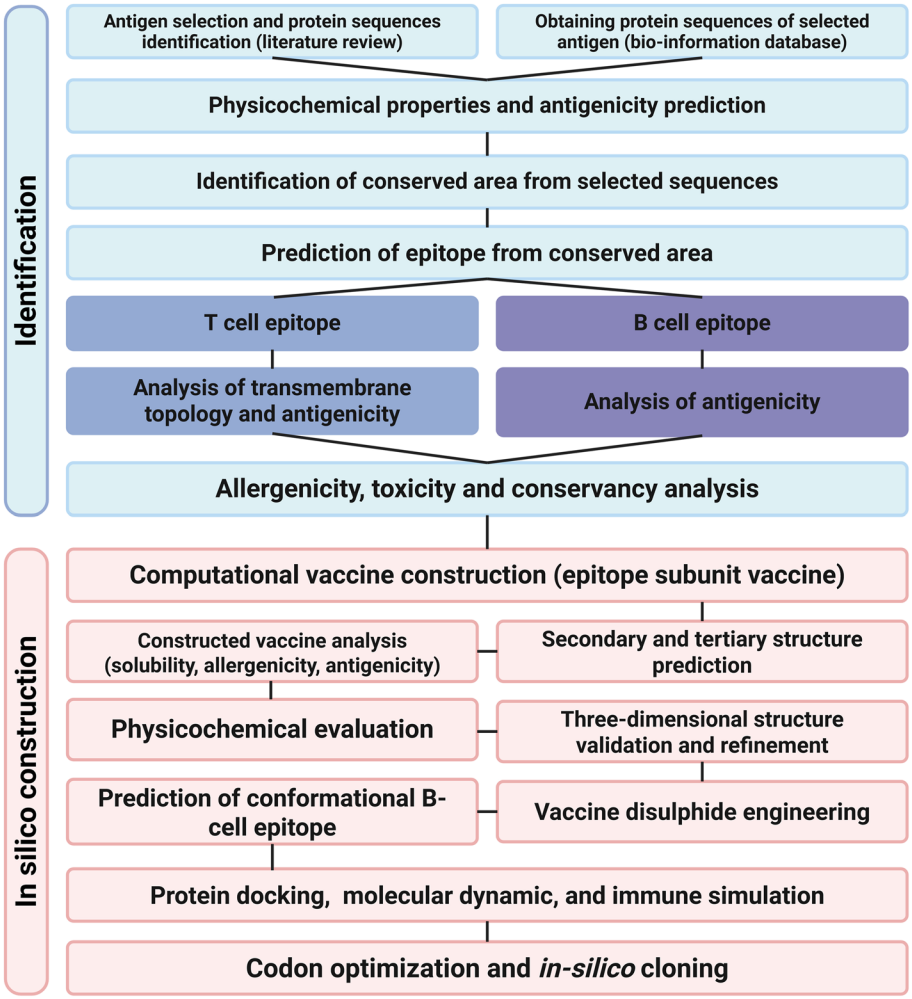

Fig. 2 Flow diagram illustrating the process of epitope prediction and the computational design of subunit vaccines (Ahmed et al. 2023; Aziz et al. 2022; Rezaei et al., n.d.; Uddin et al. 2022)

identifying epitope-based peptides involves a methodical process that combines computational bioinformatics tools, immunoinformatics, and experimental validation (Fig. 2). This process begins by selecting target proteins from pathogens and then applying computational approaches to assess and predict B-cell and T-cell epitopes by analyzing their antigenicity and affinity for binding to MHC molecules. Epitopes can be selected on the basis of various criteria, such as comprehensive genome screening, sequence conservation, localization within cells, affinity for MHC binding, and antigen annotation (Michel-Todó et al. 2020). Moreover, various computational approaches (in silico methods), such as antigenicity testing, evaluating allergies and toxicity, analyzing sequence conservation, and examining transmembranes, can provide valuable insights for identifying the most effective epitopes (Uddin et al. 2022).

The antigenic peptide/protein expression system has been widely developed to obtain efficient production on a large scale and improve the stability and conformation of peptides. For example, with respect to the expression of the classical swine fever virus (CSFV) E2 protein, various expression systems with different optimizations have been investigated. Expressing the entire length of the CSFV E2 recombinant protein is difficult. However, some studies using expression systems, such as the Pseudorabies virus (PRV) viral vector and insect cell-baculovirus expression systems, successfully expressed the entire CSFV E2 protein (Sun et al. 2023; L. Yang et al. 2017). Other expression systems, such as insect cell-baculovirus, HEK293T cells, CHO cells, E. *coli,* and yeast extraction systems, also successfully expressed truncated E2 protein (Feng et al. 2020; D. Li et al. 2020; Y. Zhang et al. 2023a, b; Zhong et al. 2024; Zhou et al. 2011). Several modifications can be made to optimize protein expression. Feng

modified the use of a Txnip promoter and a combination of 0.1 mM NADH and 0.1 mM ATP in the expression system of CHO transgenic mammalian cells (Feng et al. 2020). The modified results revealed a balance between viability cell density and production scale compared with the use of a common cytomegalovirus (CMV) promoter, which has lower productivity and low viable cell density, which is likely due to the production of cytotoxic antigenic proteins. Moreover, Yang successfully modified the insect cell–baculovirus expression system through vector modification by adding a melittin signal peptide to secrete proteins from insect cells and simplifying the purification process (L. Yang et al. 2017).

Foot-and-mouth disease virus (FMDV) VP1 protein can also be expressed via conventional expression systems such as E. *coli*, yeast, and insect cell-baculovirus expression systems (Kazemi et al. 2022; Le et al. 2024; X. Liu et al. 2017a, b; Mamabolo et al. 2020). Rao conducted research and successfully expressed the FMDV VP1 protein *via* a plant expression system in which Agrobacterium was inserted with a vector to infect sunn hemp plants (Rao et al. 2012). Plant expression systems for producing antigenic peptides/proteins in animal vaccines can produce plants with multiple functions, such as feed and vaccines, without further processing.

Vaccine development using epitope-based peptides for the treatment of animal diseases has shown varying levels of efficacy. T epitope-based vaccines primarily aim to improve cellular immunity by targeting specific T-cell responses, enhancing the generation of memory T cells, and ensuring long-lasting immunity. However, these vaccines may not always provide sufficient protection, as they do not induce neutralizing antibodies. On the other hand, B epitope-based vaccines focus on boosting humoral immunity by stimulating antibody formation and are particularly effective against infections where neutralizing antibodies are crucial. Nevertheless, they may not always generate the significant cellular immune responses necessary for eliminating intracellular infections (Blanco et al. 2013).

The incorporation of T and B-cell epitopes in vaccine formulations has achieved promising results in combating animal diseases. This method, known as multiepitope vaccination, can trigger both humoral and cellular immune responses, possibly enhancing the range of protection (Forner et al. 2021; Q. Li et al. 2023a, b). Multiple epitope approaches have been applied in FMDV vaccines. Researchers have reported that combining T and B-cell epitopes can significantly influence the immune response. They reported that peptides with a B-cell epitope placed at the N-terminus followed by the T-cell epitope were more effective at producing secondary antibodies and promoting Th1-type immunity. Furthermore, interest in multiepitope vaccines aimed at various animal diseases, such as goatpox, lumpy skin disease, and infectious bursal disease, is increasing (Dey et al. 2023; Kar et al. 2022; Long et al. 2023). Epitope-based vaccines provide several benefits, including improved safety, targeted immune responses to specific epitopes, and the potential to defend against various strains or serotypes of pathogens. Table 2 summarizes several studies related to antigenic peptides.

Immune modulation peptides

Immune modulation peptides, including ligand-conjugating peptides and adjuvant-like peptides, are designed to significantly improve immune responses, especially in combination with vaccines. Ligand-conjugating peptides are designed to be connected to receptor ligands to target specific PRRs. Receptor ligands, especially TLR agonists, act as potent adjuvants because of their ability to modulate the innate immune response (T. Zhao et al. 2023). TLR agonists have been used as potent adjuvants in vaccine formulations against infectious diseases. Studies have shown that CpG ODN (TLR agonist) can induce cellular and humoral immune responses, leading to reduced symptoms and increased survival rates against pathogens (Kayesh et al. 2023). Recently, studies have discovered adjuvant-like peptides that do not possess specific target receptors due to a lack of ligand or receptor agonists. However, these peptides have immunomodulatory effects similar to those of conventional adjuvants (Cai et al. 2014; C. Wang et al. 2008).

Compared with free ligand/agonist molecules, ligand-conjugated peptides have greater adjuvant effects. For example, TLR7 agonist–nanoparticle conjugates have been shown to significantly increase the immune response, cellular uptake, and APC activation. Furthermore, viral challenge has shown good protection in mice against different strains of SARS-CoV- 2 (Hanagata 2017; Yin et al. 2023). Another study demonstrated that TLR agonists in combination with PLGA nanoparticles and the SAG1 protein of *T. gondii* increased the humoral response and cellular response (higher IL- 2, IFN-γ, and TNF-α levels), leading to a reduction in the number of brain cysts in mice after oral challenge (Allahyari et al. 2022).

Immune modulation peptides have been developed to induce improved innate immune responses in combination with vaccines against several animal and zoonotic diseases. The data concerning recent immune modulation peptides in the veterinary field are shown in Table 3. Immune modulation peptides and targeting peptides can together generate immunomodulatory effects and amplify antigen presentation and processing. This combination may result in the maturation and activation of

Table 2 Antigenic epitope peptides

Target antigen	Target gene sequences	Epitope type	Research progress	Experiment output	Source
CSFV (swine)	Glycoprotein E2	B-cell linear single epitope	In vivo experiment (vaccination and challenge)	- Single epitope-E2 fusion showed a significant increase of antibody anti-CSFV in pig and rabbit - Provided complete protection in rabbits against HCLV - Provided partial protection in pigs against virulent CSFV (40%)	(S. Liu et al. 2006a, b; S. Liu et al. 2006a, b)
		B-cell linear quadruple epitope		- Displayed strong immuno-reactivity with both anti-CSFV and anti-E2 sera - Provided complete protection in rabbits against HCLV - Provided complete protection in pigs against virulent CSFV (100%)	
CyHV-1 (fish)	1. ORF-25 2. ORF 136B 3. Major capsid 1. Capsid triplex 2. ORF104 3. Glycoprotein	Multiepitope (B and T cell)	In-silico immune simulation	- Expected to induce a primary immune response - Expected to increase the concentration of helper T cells, CTLs, and plasma B cells - Expected to produce certain cytokines (IL- 23, IL- 10, IFN-γ, IFN-β)	(Rani et al. 2024)
JEV (swine)	Envelope (E) protein	Multiple-epitope (B-cell and T-cell epitopes)	In vivoexperiment (vaccination and challenge)	- Multiepitope-based vaccine induces a robust antibody response. Producing antibody levels similar to the inactivated JEV vaccine - Provided significant cytokine responses (IL- 4 and IFN-γ) that were comparable to the control vaccines at the highest dose - Induced high Nab titers - Provided complete protection (100%) in mice against lethal JEV	(F. Wang et al. 2012; J. Wei et al. 2010)

Table 2 (continued)

Target antigen	Target gene sequences	Epitope type	Research progress	Experiment output	Source
ASFV (swine)	E184L protein	Linear B-cell epitopes	Epitope identification using monoclonal antibodies E184L (A10, 2D2, 3H6, and 4C10)	Identification of two highly conserved linear B-cell epitopes of the E184L protein across different ASFV isolates - 119-IQRQGFL- 125 recognized by mAb 1 A10 - 153-DPTEFF- 158 recognized by mAbs 2D2, 3H6, and 4C10	(Tesfagaber et al. 2024)
PDCov (swine)	Structural proteins - Spike (S) - Envelope (E) - Membrane (M) - Nucleocapsid (N))	T-cell epitopes, specifically SLA I-specific epitopes (swine leukocyte antigen class I)	Epitope identification and in vivo experiment	- Induced strong cellular immune responses specific to the epitope - Stimulated IFN-γ production in vaccinated piglets	(Wen et al. 2023)
FMDV (swine and cattle)	VP1 protein (serotype A)	Linear B-cell epitopes	Epitope identification using - mAb 949	- Identification of linear neutralizing B-cell epitopes of the VP1 protein - The region comprising residues 143–153 is highly conserved among different strains of serotype A FMDV	(Liang et al. 2016; W. Liu et al. 2017a, b; Ru et al. 2023; D. Yang et al. 2011)
		Conformational B-cell epitope	- mAb 6C9	Residues 135-YxxPxxxxxGDLG-147	
	VP1 protein (serotype O)	Linear B-cell epitopes	- mAb 8E8	The region comprising residues 145 to 154 is highly conserved among different strains of serotype O FMDV	
	VP2 protein (serotype O)	Conformational B-cell epitope	- mAb 3D9	Residues 89-GVYxxxxxxx-AYxxxxW- 105	
PEDV (swine)	Spike (S) protein	Conformational B-cell epitopes	Epitope identification using neutralizing monoclonal antibodies (nmAb)	Identification of two conformational neutralizing B-cell epitopes of PEDV S protein - 576–639 aa (C-terminus of COE epitope) - 435–485 aa (S1 A domain)	(Chang et al. 2019)

Table 2 (continued)

Target antigen	Target gene sequences	Epitope type	Research progress	Experiment output	Source
Toxoplasma gondii (parasite)	ROP8 protein	Multiple-epitope (B and T-cell epitopes)	In vivo experiment (vaccination and challenge)	- Demonstrated a notable increase in anti-*T. gondii* antibody levels compared to the control groups - Exhibited a combined IgG1/IgG2a immune response, with a higher production of IgG2a - Vaccinated mice showed an increase in IFN-γ production, suggesting the activation of a Th1-type cellular immune response - Vaccinated mice experienced extended survival periods following exposure to *T. gondii* in comparison to the control groups	(Foroutan et al. 2020)
Tembusu virus (duck)	Envelope (E) protein	Multiepitope (B and T-cell epitopes)	In vivo experiment in duck (vaccination and challenge)	Multiple epitopes significantly outperformed the control group in generating TMUV-specific antibody responses - The average neutralizing antibody titer in the vaccinated group reached 1:16, compared to a complete lack of such antibodies in the control group - The survival rate in the group that received the vaccine was more than double that of the control group, standing at 70% versus 30%	(Han et al. 2016)
IBV (poultry)	- Spike (S) protein - Nucleocapsid (N) protein	Multiepitope (B and T-cell epitopes)	In vivo experiment in chicken (vaccination and challenge)	- Multiepitope vaccine provided 80%– 100% protection against the IBV challenge, compared to 0% in control groups - Higher antibody titers and stronger CD8+T-cell proliferation response - Induced both neutralizing antibodies and cellular immune responses	(Tan et al. 2016; T. Yang et al. 2009)

Table 2 (continued)

Target antigen	Target gene sequences	Epitope type	Research progress	Experiment output	Source
Anthrax (bacteria) (cattle)	- Domain 4 of protective antigen (PA) - N-terminal of lethal factor (LFn)	Multiepitope (B and T-cell epitopes)	In vivo experiment (vaccination and challenge)	- Combination of T and B epitope showed comparable immunogenicity and protective efficacy to full-length PA protein - Elicited mixed Th1-Th2 immune responses - pProvided 80% protection in mice against challenges with virulent *B. anthracis* spores similar to full-length PA	(Aggarwal et al. 2019)
Rabies virus	G protein	Linear neutralizing B-cell epitopes	In vivo experiment (vaccination and challenge) in mice and dog	- Induced neutralizing antibody titers of 9 IU/mL in dogs and 5 IU/mL in mice (28 dpi) - Showed 70–80% survival rate in mice	(Niu et al. 2016)

Abbreviations: *CSFV* classical swine fever virus, *CyHV* cyprinid herpesvirus, *JEV* Japanese encephalitis virus, *ASFV* african swine fever virus, *PDCov* porcine deltacoronavirus, *FMDV* foot and mouth disease virus, *PEDV* porcine epidemic diarrhea virus, *TMUV* tembusu virus, *IBV* infectious bronchitis, *ORF* open reading frame, *CTL* cytotoxic T cell, *IL* Interleukin, *IFN* interferon, *ROP* rhoptry protein, *VP* viral protein, *PA* protective antigen, *G* glycoprotein

Table 3 Immune modulation peptides

Type	Peptide/protein composition	Target receptor	Pathogen	Outcome	Source
Ligand-conjugated peptides	IBV S protein+ Flagellin (TLR-5 ligand) SAPN	TLR-5	Infectious bronchitis virus (poultry)	Flagellin enhanced both humoral and cellular immune responses without additional external adjuvant	(J. Li et al. 2018a, b)
	M2e+ Helix2 H5 N2 coassembly with flagellin (TLR-5 ligand)		H5 N2 Avian Influenza	- Induced a higher level of antibody - Increased cross-neutralizing activity	(Karch et al. 2017)
	FMP014 SAPN+ flagellin		*Plasmodium falciparum*	Increased cytokine release and immunomodulatory effect	(Kaba et al. 2018; Qian et al. 2015)
	PRRSV GP5 (glycoprotein) protein+ fljB (flagellin)		PRRSV	Enhanced humoral immune response	(Xiong et al. 2015)
	PCV2 Cap VLP+ CSFV E2 protein	B-cell receptors	CSFV	- Enhanced antigen captured by APCs - Increased APC maturation - Increased cytokine production	(Z.-H. Liu et al. 2022a, b)
Adjuvant-like peptide	Cross-ß nano assemblies + M2e protein	- No specific target receptor - Activating TLR- 2/6	Influenza A virus	- Increased specific antibody response - Increased innate immunity	(Al-Halifa et al. 2020)
	Recombinant protein consists of: - Thioredoxin (TRX) - Bursin (K-H-G) - Analog bursin (T-X-N-L)	- No specific target receptor - Stimulating B cells	Japanese encephalitis virus	- Stimuated Th1 and Th2 responses - Promoted B-cell proliferation	(Cai et al. 2014; C. Wang et al. 2008)
	Hybrid polypeptides consist of: - Bursin (K-H-G) - Gagnon (K-E-P-Y)		Infectious bursal disease virus		
	Thymosin α−1 (Tα1) in combination with E2 protein	- No specific target receptor - Stimulating T cells	CSFV	- Triggered T-cell maturation - Promoted lymphokines expression	(Y.-G. Xu et al. 2015)
	Recombinant porcine IFN-ɣ	- No specific target receptor - Stimulating T cells	PCV2	- Enhanced T lymphocyte proliferation - Induced humoral immune response	(Melgoza-González et al. 2023)
	Recombinant flagellin (rFLA)	TLR-5	*Piscirickettsia salmonis*	- Upregulated pro-inflammatory cytokine expression - Activated T-cell	(González-Stegmaier et al. 2021)
			H5 N1 AIV	Increased cell-mediated responses	(St Paul et al. 2014)
	Pam3 CSK4 (lipopeptides)	TLR-2	H5 N1 AIV	Increased cell-mediated responses	

Abbreviations: *PRRSV* porcine reproductive and respiratory syndrome virus, *AIV* avian influenza virus, *CSFV* classical swine fever virus, *PCV2* porcine circovirus 2, *SAPN* self-assembling protein nanoparticle, *IBV* infectious bronchitis, *VLP* virus-like particle, *TLR* toll-like receptor, *IFN* interferon

APCs, the synthesis of proinflammatory cytokines, and an increase in costimulatory molecules. Furthermore, it augments both humoral and cellular immune responses. Collectively, these effects form a potent integration that

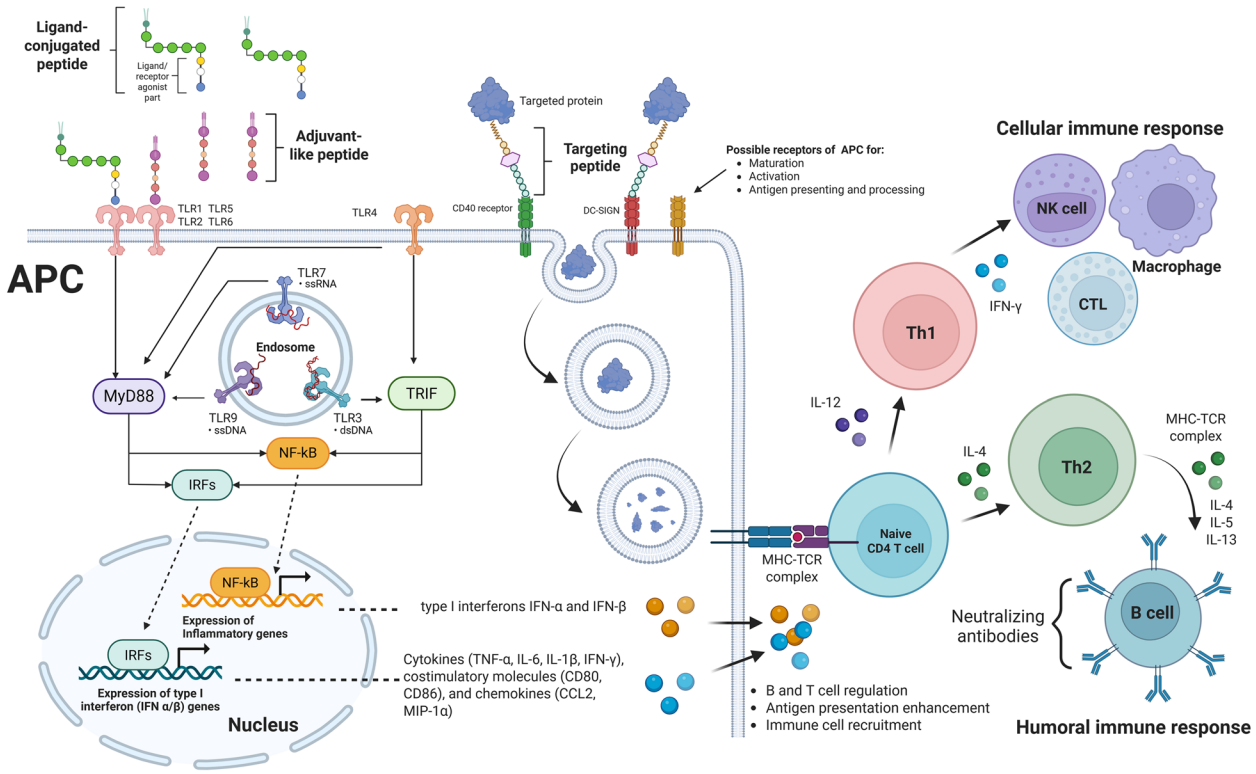

Fig. 3 The possible collaborative functions of targeting peptides and immune modulation peptides enhance both the immunogenicity and presentation of antigens

enhances the immunogenicity and precise targeting of vaccines (Fig. 3) (Luchner et al. 2021; Reed et al. 2013).

Like antigenic peptides, immune modulation peptides utilize multiple techniques to produce functional peptides or proteins. The *E. coli* expression system is the most frequently used method. This system is designed to achieve a high protein yield through a simple purification process, effectively characterizing peptide or protein expression results. Some research indicates that this expression system often needs optimization, particularly when insoluble peptides or proteins are expressed. To increase solubility, facilitate coassembly, and aid in refolding, additional protocols, which may include the application of a lysis buffer (8 M urea, 100 mM NaH_2PO_4, 5 mM tris (2-carboxyethyl) phosphine (TCEP), 5% glycerol, 20 mM Tris, pH 8.0), are essential (González-Stegmaier et al. 2021; Kaba et al. 2018; Karch et al. 2017; J. Li et al. 2018a, b; Qian et al. 2015; Rao et al. 2012; Xiong et al. 2015). Moreover, several studies have indicated the use of a combination of other systems, such as that conducted by Al-Halifa, which employed solid-phase peptide synthesis (SPPS) in conjunction with HCTU (Al-Halifa et al. 2020). High-performance liquid chromatography (HPLC) was utilized in the purification process. The chimeric M2e peptide produced through this expression process exhibited better characterization and conformation. Xu successfully produced thymosin α−1 *via* the Lactobacillus plantarum bacterial expression method (Xu et al. 2015). This technique allows for the production of peptides with enhanced immunogenicity. These findings underscore the synergistic potential of immune modulation peptides in vaccine design, which will be further explored in the context of delivery systems.

Delivery peptides

Targeting peptides/polypeptides

Targeting peptides/polypeptides are biomolecules engineered to bind specific receptors/antigens, enabling precise therapeutic intervention. Targeting peptides exhibit high specificity and selectivity for their target proteins (antigen uptake), minimizing off-target effects and reducing potential side effects (Rossino et al. 2023; Todaro et al. 2023). The small size of the targeting peptides and polypeptides enhances their ability to penetrate tissues and disperse across the body more effectively. Additionally, their potential for chemical modifications can increase their bioavailability and stability, addressing usual issues such as degradation and short half-life (M. Liu et al. 2021a, b).

Advanced techniques are utilized to carefully choose and develop peptides or polypeptides that are directed at specific targets. Techniques such as phage display are used swiftly to find optimal matches by using bacteriophages to screen target receptors (S. Ma et al. 2019; R. Ouyang et al. 2024). Alternative approaches, such as the use of yeast, mammalian expression systems (HEK293T cells and CHO cells), and bacterial expression systems (*Lactobacillus plantarum* and E. *coli*), can produce both molecules aimed at targeting and the receptors or proteins with which they bind (Y. Jiang et al. 2015; D. Li et al. 2020; Pastor et al. 2024; Zhu et al. 2023). Moreover, the use of computational design plays a crucial role in deciphering the interactions between these targeting peptides and their intended targets, significantly enhancing the efficiency and effectiveness of the process (Aloisio et al. 2021; Jefferson et al. 2023). Current studies have demonstrated notable advancements in the use of targeting peptides/polypeptides for the treatment of animal diseases. The primary application of these peptides/polypeptides in the field of veterinary medicine includes focusing on peptides that target antigen-presenting cells (APCs) and peptides aimed at various other cell membrane receptors (Fig. 4).

In a recent study, Xia successfully identified a new peptide known as HS (HSLRHDYGYPGH) that targets dendritic cells by using a phage-displayed peptide library (Xia et al. 2024). When this peptide was integrated into a recombinant Lactobacillus strain that expresses the VP60 capsid protein of rabbit hemorrhagic disease virus rabbit hemorrhagic disease virus (RDHV), this peptide significantly increased the ability of rabbit dendritic cells to capture RHDV and enhanced immune responses. In another study, introduced nanobody peptide conjugates (NPCs), which integrate PRRSV-specific nanobodies with peptides derived from CD163 receptors (Yang et al. 2024). These NPCs have shown great effectiveness against a variety of PRRSV strains by preventing viral proteins from attaching to CD163 receptors. Information on the latest advancements in targeting peptides for animal disease research can be found in Table 4.

Cell-penetrating peptides (CPPs)

CPPs are short peptide sequences consisting of fewer than 50 amino acids that can cross and internalize cell membranes through several mechanisms, including indirect endocytosis and direct entry. They can also transport various molecular cargoes (nanoparticles and liposomes), drugs, nucleic acids, and specific proteins or peptides into cells (Fig. 5). Recently, CPPs have been used in antitumor treatment, vaccine development, and gene therapy (Rádis-Baptista 2021; Robledo et al. 2023; H. Zhang et al. 2023a, b). In veterinary medicine, CPPs can be derived from animal viruses and used to develop certain vaccines against animal diseases.

Various methods can be used to acquire cell-penetrating peptides. These methods include computational design and screening, phage display with biopanning, and several strategies, including cyclic CPPs, glycosylated CPPs, chimeric CPPs, and D-form CPPs. The potential of natural sources such as venom, microbes, and plants to yield cell-penetrating peptides has also been explored (J et al. 2024; J. Ouyang et al. 2022; Park et al. 2023). Moreover, Adhikari successfully produced cell-penetrating peptides attached to protein cargoes *via* the *E. coli* expression system (Adhikari et al. 2018). The *E. coli* expression system is a potential method for CPP expression because of its good solubility; it does not require treatment with a refolding buffer or denaturant, facilitating production with high scalability and efficient purification (Kang et al. 2018; G. Zhang et al. 2021a, b, c).

The penetration mechanism of CPPs remains unclear. However, the mechanism is divided into direct penetration and endocytosis. Direct penetration occurs when peptides cross the plasma membrane without requiring energy. This can occur through pore formation, inverted micelle formation, or the carpet model. Uptake via endocytosis encompasses many routes, including macropinocytosis, caveolae-mediated endocytosis, and clathrin-mediated endocytosis (Madani et al. 2011). CPPs have shown the capacity to augment immunogenicity when integrated with nanoparticles, nucleotides, drugs, and proteins and to improve targeted delivery and cellular absorption, potentially resulting in more robust immune responses. Moreover, CPPs accumulate more in antigen-presenting cells, thereby increasing T-cell priming and activation in vivo. These improvements are confirmed by significant increases in CD8+ T-cell responses following immunization with antigens conjugated to CPPs (Backlund et al. 2022; Gessner & Neundorf 2020).

While preserving the biological activity of the nucleotides, CPP-conjugated siRNA has showed an extraordinary increase in distribution efficiency. This improved delivery could promote stronger immune responses against particular antigens, resulting in more successful gene silencing (Zhang et al. 2021a, b, c). By facilitating the transport of impermeable chemicals across cell membranes, CPPs also increase the bioavailability and therapeutic effectiveness of certain drugs. Therefore, this mechanism could improve drug accumulation at some

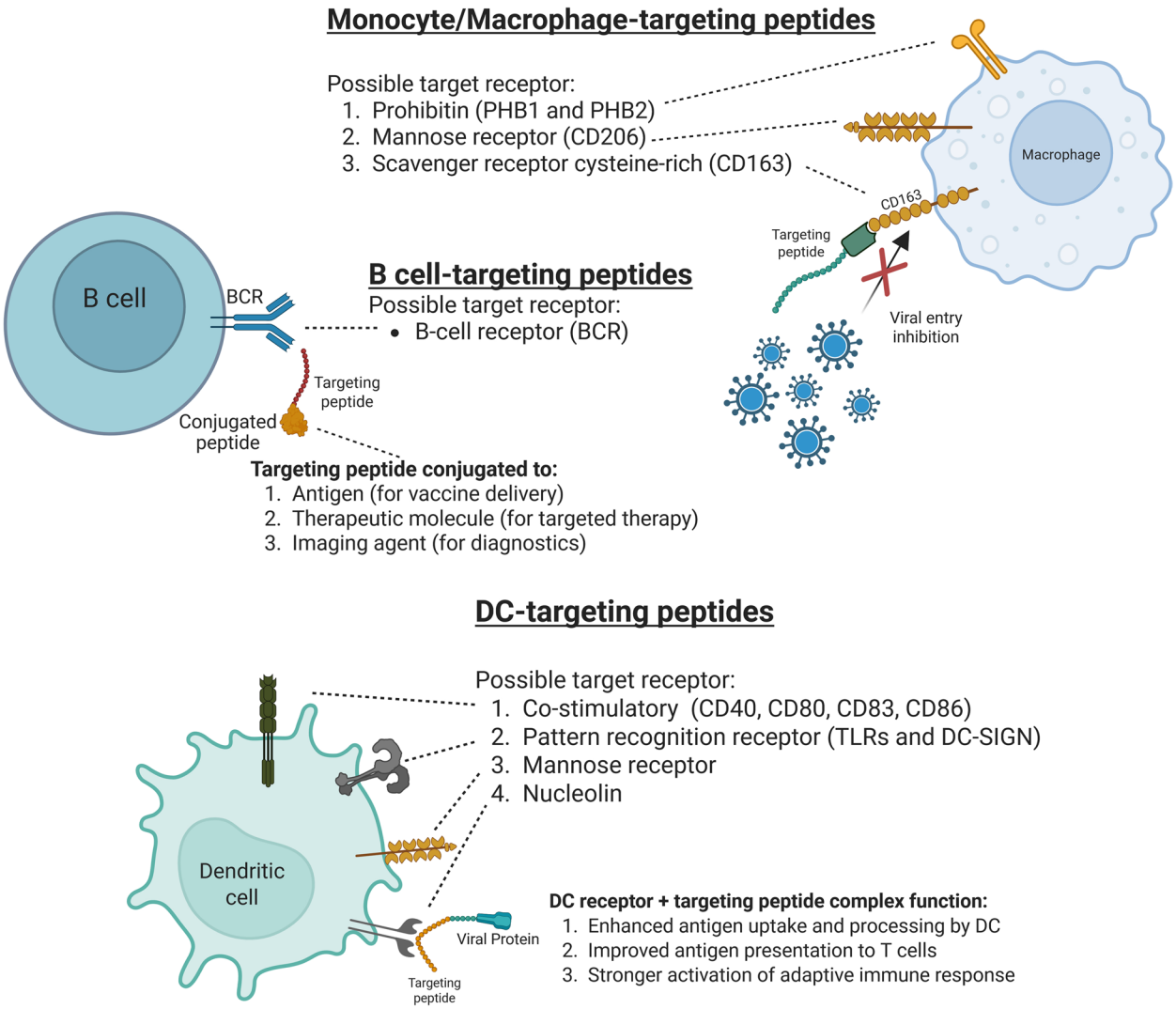

Fig. 4 Illustration of the mechanism and function of the APC-targeting peptide

locations and increase their immunogenicity (Backlund et al. 2022; Trabulo et al. 2010). The information regarding the application of CPPs in veterinary medicine is presented in Table 5.

Antimicrobial peptides

Small peptides known as antimicrobial peptides (AMPs) play a significant role in the natural immunological reactions observed in many animals. Usually, these amphiphilic peptides are positively charged, and a relatively short chain length defines them. The broad spectrum of antimicrobial activity of AMPs—including bacteria, fungi, parasites, and viruses—positions them as interesting candidates for tackling the development of antibiotic resistance (Huan et al. 2020; Rodrigues et al. 2022; R. Zhang et al. 2022). The common approaches to generate AMPs are computational design and recombinant DNA technology with bacterial expression systems (Hao et al. 2024; Hong et al. 2019). Certain antimicrobial peptides, including β-defensins and LL-37, have also been effectively produced through the *E. coli* expression system (Z. Li et al. 2018a, b). Additionally, a study by Tai successfully generated TP4 AMP via a yeast expression system (*Pichia pastoris*) to scale up recombinant protein production (Tai et al. 2021) .

AMPs can trigger an immune response through various mechanisms. For example, LL-37 AMP binds to the FPR2 receptor to recruit immune cells. Additionally, hBD3 affects STAT1 phosphorylation in T cells, influencing signaling pathway modulation (Diamond et al.

Table 4 Targeting peptides

Type	Sequence	Target receptor	Pathogen	Outcome	Source
DC-targeting peptide	FYPSYHSTPQRP	TLR4 CD80 MHC-II	H9 N2 Avian Influenza	- Activate DCs - Enhance adaptive immune response - Facilitate T and B-cell activation	(Shi et al. 2016; Sun et al. 2018)
			Transmissible gastroenteritis coronavirus (TGEV)	- Enhance antigen presentation - Stimulate T and B-cell activation	(Jin et al. 2018)
			Newcastle disease virus	- Increase IgA - Stimulate T-cell activation	(Y. Jiang et al. 2015)
Macrophage-targeting peptide	SRCR 5–9	CD163	PRRSV	Inhibit PRRSV infection by blocking virus attachment to CD163 receptor	(Deng et al. 2023; H. Yang et al. 2024; Zhu et al. 2023)
	4H7 8H2			Inhibit PRRSV infection by blocking virus attachment and replication postattachment to CD163 receptor	
DC-targeting peptide	NiV G (ECD) NiV F (aa 45–90) NiV N (aa 318–355)	CD40	Nipah virus	- Induce protective immunity and cross-neutralization against different strains - Enhance T and B-cell responses	(Pastor et al. 2024)
DC-targeting peptides	SPHLHTSSPWER	Not specified	IBDV	Enhances binding to DCs	(S. Ma et al. 2019)
DC-targeting peptides	LYPPPY (CTLA4-6 aa)	B7	PEDV	- Enhance antigen capture - T-cell activation	(Xia et al. 2022)
Macrophage-targeting peptide	Chicken heterophil CATH2 (1–13)-TP5 (CbTP) RFGRFLRKIRRFRRKDVY	TLR2	Immunosuppressive disease	Inhibit immunosuppression	(X. Wei et al. 2021)
DC-targeting peptide	HSLRHDYGYPGH KCCYPN MYPPPY	CD86 MHC-II	Rabbit hemorrhagic disease virus (RHDV)	- Enhance antigen capture - Promote DC maturation - Increase cytokine secretion	(Xia et al. 2024)

Abbreviations: *PRRSV* porcine reproductive and respiratory syndrome virus, *PEDV* porcine epidemic diarrhea virus, *IBDV* infectious ursal disease virus, *TLR* toll-like receptor, *DC* dendritic cell, *MHC* major histocompatibility complex, *IG immunoglobulin*

2009; Duarte-Mata & Salinas-Carmona 2023; H. Li et al. 2023a, b). Furthermore, AMPs can activate and differentiate cells and neutralize endotoxins. Zhang reported that LL-37 AMP can recruit neutrophils, NK cells, and mast cells (Zhang et al. 2021a, b, c), whereas Diamond reported that both α- and β-defensins AMP can modulate cell recruitment and cytokine release (Diamond et al. 2009). The use of AMPs in veterinary medicine can be categorized depending on their source, whether they are of animal or nonanimal origin or utilized for animal treatment. Many AMPs have been found in domestic animals, livestock, and poultry, indicating their essential function in the immune defense system of these species against certain infections. AMPs act through diverse mechanisms: membrane disruption, intracellular targeting, ion sequestration, biofilm inhibition, immunomodulation, and synergy with antibiotics (Fig. 6) (Kumar et al. 2020; Zhang et al. 2021a, b, c). These benefits include better biocompatibility, lower host cell toxicity, and the potential to develop treatment plans tailored to various species (Rodrigues et al. 2022; Saeed et al. 2022; Valdez-Miramontes et al. 2021).

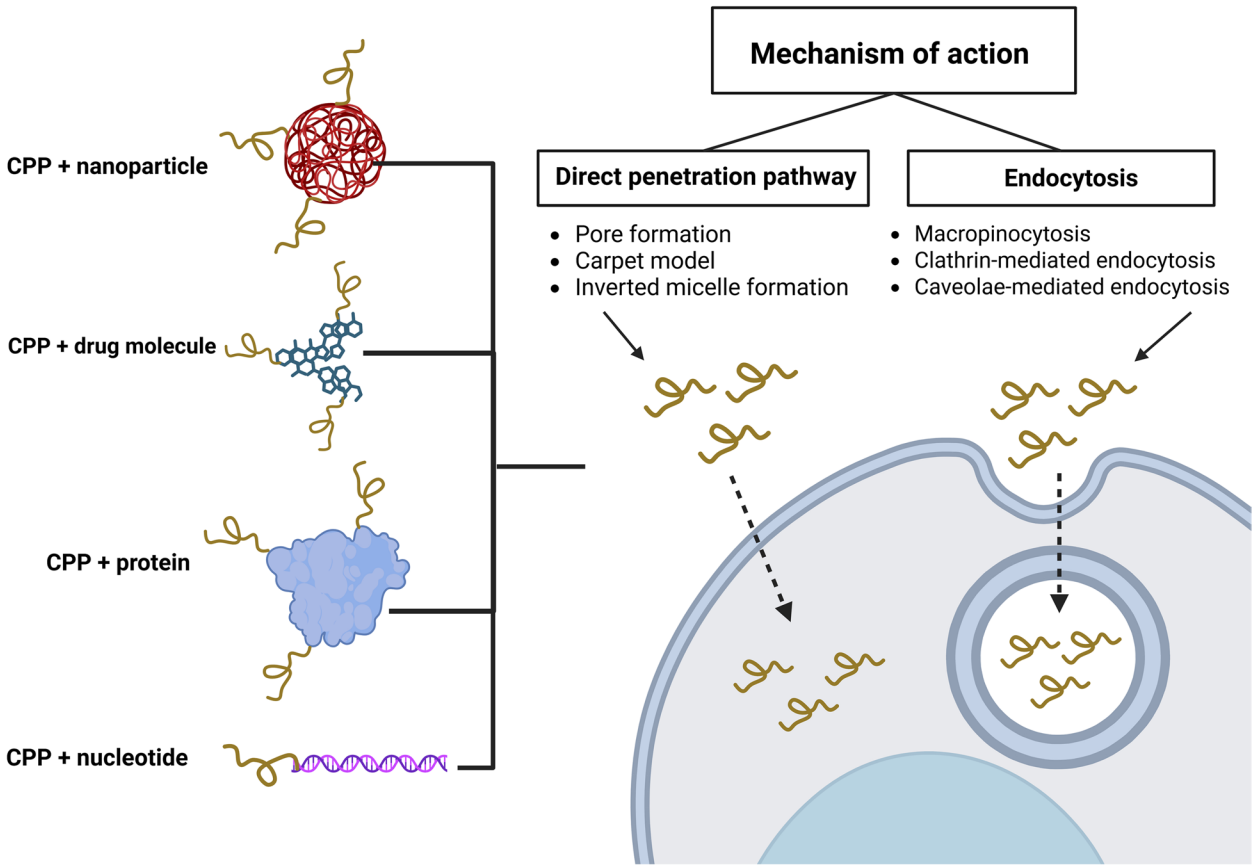

Fig. 5 CPP application and cell penetration mechanism

Moreover, the existence of AMPs in animals shows that these peptides have been developed to fight certain infections common to their surroundings efficiently. This evolutionary quality might provide a competitive edge against veterinary-related infections. In veterinary medicine, AMPs offer a possible substitute for traditional antibiotics. This strategy improves the health and output of livestock, hence supporting the sustainability of the world livestock sector and perhaps reducing antibiotic resistance (Kumar et al. 2020; Valdez-Miramontes et al. 2021). Table 6 shows the antimicrobial peptide data.

Conclusion and perspective

Functional immunopeptides mark a significant advancement in veterinary medicine for preventing and treating animal diseases. Peptides for antigenic, antibacterial, and immunological regulation and delivery have demonstrated remarkable potential in enhancing therapeutic treatments and vaccination efficacy. These peptide-based solutions provide extraordinary adaptability, better selectivity, increased immunogenicity, and stronger delivery capacity than conventional techniques do. Future advancements may involve integrated systems combining multiple peptides.

It is essential to highlight strategies that can amplify peptide conformation and structural integrity through certain modifications. These strategies include the introduction of disulfide bonds, thioamide bonds, or chemical cross-linking agents such as glutaraldehyde, carbodiimide, and transcarbamylase to effectively maintain the stability of peptides and reduce peptide degradation and cleavage by proteases (Alavarse et al. 2022; Bhardwaj et al. 2016; Habermann & Murphy 1996; A. Liu et al. 2021a, b). Moreover, replacing specific amino acids in the target peptide with unusual amino acids helps prevent protease detection and destruction of the peptide. For example, replacing L-amino acids with D-amino acids in short peptides can significantly increase peptide stability (Miller et al. 1995). Methylation of the N-terminus of peptides—substituting one or more NH groups in the

Table 5 Cell-penetrating peptides

CPP Peptide	CPP Sequence	Conjugation	Target Cell/Pathogen	Outcome	Source
TAT (HIV-1)	GRKKRRQRRRYK	Peptide nucleic acids (PNA) = Anti-gyrase A subunit gene (ttgcattatatg)	*Streptococcus suis* (swine bacteria)	Bactericidal effect	(Zhu et al. 2024)
NLS-A (PCV2)	MTYPRRRFRRRRHRPRS	-	PK15, HeLa, NIH3 T3	Novel CPP peptides derived from PCV2	(Yu et al. 2018)
- CPP5 - TAT	- KLVPM - YGRKKRRQRRR	Cre recombinase protein	Porcine fetal fibroblasts (PFFs)	Translocation of Cre protein across the plasma membrane of PFFs (transgenic pig)	(Kang et al. 2018)
Z12 (zebra protein)	KRYKNRVASRKCRAKFKQLLQHYREVAAAKSSENDRLRLLLK	ASFV protein p30, p54, and T-cell epitope	RAW264.7 cells	- Increase cellular uptake - Induce neutralizing antibodies and good immunogenicity	(G. Zhang et al. 2021a, b, c)
VG21 (vesicular stomatitis virus)	VTPHHVLVDEYTGEWVDSQFK	Gold nanoparticles (GNPs)	Vero Hep-2 Cos-7 HeLa	Enhance cell internalization and biodistribution of gold nanoparticles	(Tiwari et al. 2014)
Penetratin (PEN)	RQIKIWFQNR RMKWKK	Porcine scFv (transbodies) against NSP1β-PRRSV	MARC-145	Increase cell internalization efficiency	(Thueng-In et al. 2023)
- CPP1 - CPP2 (BFDV)	- RRYRRRRRYFRKRR - RRRRYARPYYRRR	-	DF-1 cells	CPP1 and 2 derived from beak and feather disease (BFDV) increase entry efficiency	(Sitinjak et al. 2023)
CD2v (ASFV)	KPCPPP	-	CHO (chinese hamster ovary)	Provide CHO cell internalization	(S. Yang et al. 2021)
CVP1-N2 (chicken anemia virus)	LKRLRRRYKFRHRRRQRYRRR	Apoptin gene	HCT116	Intracellular delivery vehicle	(Hu et al. 2020)
HA314 - 46 (HA protein of H5 N1 HPAIV)	TIGECPKYVKSNRLVLATGLRNSPQRERRRKKR	-	KU812 COS-7	Increase cell internalization	(Kajiwara et al. 2020)
AAV.CPP.16 (adeno-associated virus from macaques)	TVSALK	-	- HEK293T - Blood–brain barrier	Provide blood–brain barrier penetration in nonhuman primates	(Yao et al. 2022)

Abbreviations: *HIV* human immunodeficiency virus, *TAT* transactivator of transcription, *NLS* nuclear localization signal, *PCV2* porcine circovirus 2, *CPP* cell-penetrating protein, *ASFV* african swine fever virus, *HPAIV* highly pathogenic avian influenza

peptide backbone with N-methyl groups—has also been shown to improve peptide stability (Linde et al. 2008). Owing to the reversible and irreversible unfolding of proteins that occurs during lyophilization, they can adopt conformations that are susceptible to degradation by proteolytic enzymes during storage. This method reduces the physiological qualities of proteins (Moorthy et al. 2015). The addition of cryoprotectants such as sucrose, glycerol, and alginate to lyophilizers greatly increases the storage duration of peptide fragments (Gorka et al. 2020; Karunnanithy et al. 2024; J. Li et al. 2024). The efficient construction of antimicrobial peptides is made possible by the coordinated production of certain peptides that selectively attach to cellular receptors and immunomodulatory peptides at the genomic level. Molecular connections, including SpyTag at one end, and delivery mechanisms, including SpyCatcher tags, help us to enable peptide–protein interactions effectively. This strategy allows the use of synergistic benefits such as exact delivery, immune modulation, and direct antibacterial effects.

Through the development of artificial intelligence deep neural networks, researchers have been able to predict and design targeted peptides for receptors of interest, e.g., the UniPMT framework developed by Zhao is capable of predicting MHC and T-cell receptor (TCR)-binding peptides (Y. Zhao et al. 2025), and Kirsten Dietze-Schwonberg et al. have been able to predict peptides by

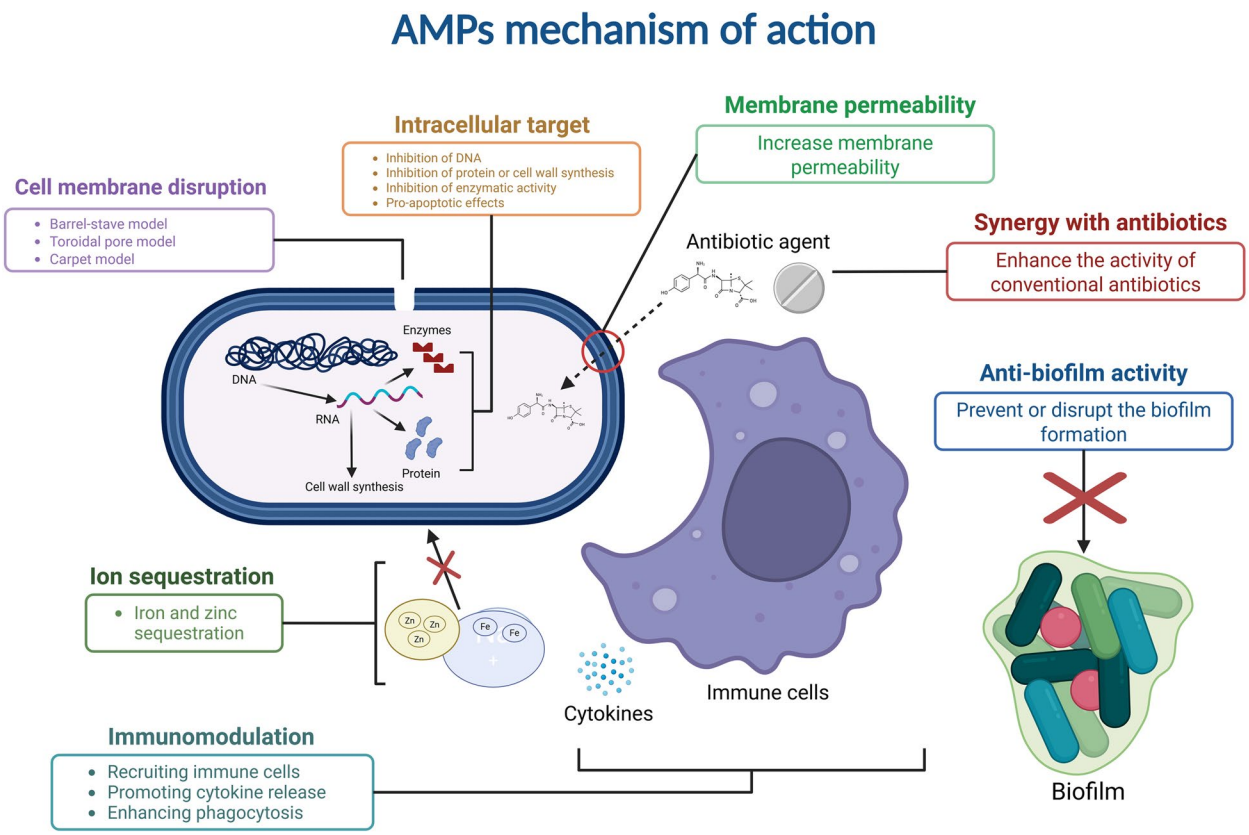

Fig. 6 Antimicrobial peptide mechanism of action

predicting CD 8+ epitope peptides specific for Leishmania major in mice *via* the SYFPEITHI algorithm. The peptides targeted by computers have strong potential as vaccine candidates (Dietze-Schwonberg et al. 2017). Sun et al. analyzed PRRSV via NetMHCpan4.1, IEDB, Alphafold and other artificial intelligence software, and analyzed the structural proteins of the PRRSV NADC30-like strain, which are predicted to be functional peptides that can significantly stimulate the immune response of B and T cells, and introduced the SpyCatcher system to display this antigenic protein on the surface of the nanoparticles to demonstrate good immunogenicity and protection in vitro and in vivo experiments (Sun et al. 2025). The above strategies will help address the current limitations in protein stability, bioavailability, immunogenicity, and cellular uptake while providing more targeted and effective therapeutic strategies.

The ability to scale up production and maintain cost-effectiveness will become increasingly important. Ensuring the economic viability of peptide-based techniques for broad use in veterinary practice will depend on developments in production technology, especially enhanced recombinant expression systems. Investigating emerging diseases also offers a great opportunity. For instance, the ASFV has caused significant harm to the worldwide swine industry. Although no vaccine has yet been developed against the virus, researchers can identify conserved peptides of ASFV antigenic proteins, e.g., by screening and analyzing monoclonals capable of target-binding ASFV E184L antigenic proteins, Tesfagaber reported that two of the linear epitopes of the E184L antibody (^{119}IQRQGFL^{125} and ^{153}DPTEFF^{158}) are highly conserved among different ASFV isolates (Tesfagaber et al. 2024). The development of a subunit vaccine targeting this conserved peptide will provide new insights into solutions for the clearance of this virus. Additionally, for highly pathogenic avian influenza (HPAI), a zoonotic disease that poses a serious threat to global public health, studies have been conducted to develop nanovaccines on the basis of the immunogenic epitope of the extracellular domain of the matrix protein 2 of influenza A viruses (M2e) (Al-Halifa et al. 2020). Since this M2e peptide is highly conserved among different influenza A virus strains, coupling this peptide in fibrous nanoparticles capable of self-assembly can trigger a strong immune response in model mice.

Table 6 Antimicrobial peptides

AMP Name	Source	Structural Class	Target Pathogen/Activity	Mechanism of Action	Source
LFchimera	Bovine (derived from bovine lactoferrin)	α-helixes (chimeric peptide)	- Broad-spectrum gram-negative and positive bacteria - Fungi - Parasite - Biowarfare agents (*Bacillus anthracis, Yersinia pestis*, *Burkholderia pseudomallei*)	- Membrane disruption/permeabilization - Intracellular targeting (for *Burkholderia pseudomallei*)	(Jean et al. 2016; Ligtenberg et al. 2021)
Bomidin	Bovine (bovine myeloid AMP-27)	α-helical	- Bacteria (12 species) - Enveloped viruses (SARS-CoV-2, HSV- 2, DENV- 2, CHIKV)	Cell membrane disruption	(R. Liu et al. 2022a, b)
- NK2 A - NK2B - NK1 - NK2C	Bovine NK-Lysin	Cationic α-helical	- Multidrug-resistant Salmonella - Mannheimia hemolytica - *Staphylococcus aureus*	Cell membrane disruption	(Dassanayake et al. 2021; N. Jiang et al. 2022)
BMAP-28	Bovine cathelicidin genes	α-helical	- Mannheimia hemolytica, BHV-1, BRSV - Methicillin-susceptible *Staphylococcus aureus* (MSSA) - Methicillin-resistant *Staphylococcus aureus* (MRSA)	Membrane permeabilization	(Bao et al. 2024; Cornejo et al. 2024; Takagi et al. 2012)
Bactenecin-5 (bac-5)		Proline-rich	*Mannheimia hemolytica*		
WK2	Derived from bacteriocin Leucocin A	β-sheet			
BSN-37	Bovine (positions 2–38aa in the Bac5 sequence)	Proline-rich	Gram-negative bacteria (e.g. *Salmonella*, *Escherichia coli*)	Cell membrane disruption	(Y. Xu et al. 2023)
Cp1	Bovine αS1-casein	Not specified	E. *coli*, *S. enterica*, S. *pullorum*, *L. monocytogenes*, *S. aureus*	Membrane disruption/permeabilization	(Hou et al. 2018)
LPcin-YK3	Bovine milk (derived from lactophoricin)	Cationic amphipathic α-helical	- Gram-positive bacteria (L. *innocua*, *S. aureus*), - Gram-negative bacteria (*P. aeruginosa*, S. *typhimurium*, E. *coli*) - Fungi (*C. albicans*)	Membrane permeabilization	(Kim et al. 2017)
Tilapia piscidin 4 (TP4)	Nile tilapia (fish)	Linear, Cationic, Histidine-rich	1. Gram-positive and gram-negative bacteria - *Escherichia coli* - *Pseudomonas aeruginosa* - *Staphylococcus aureus* - *Riemerella anatipestifer* strains 2. Enhanced phagocytosis in domestic chicken 3. Improved growth performance in domestic chicken	Cell membrane disruption	(Tai et al. 2021)

Table 6 (continued)

AMP Name	Source	Structural Class	Target Pathogen/Activity	Mechanism of Action	Source
LLV	LL-37 (human cathelicidin)	α-helical	1. Broad-spectrum (gram-negative and gram-positive bacteria, fungi, viruses) 2. Enhanced immune function in broiler chicken (dose at 100 mg/kg)	- Cell membrane disruption - Bacterial growth inhibition	(X. Liu et al. 2024)
C2-2	Chicken (derived from chicken CATH-2)	α-helical	Multidrug-resistant *Escherichia coli* (increased survival rates in MDR E. *coli*-infected chicken)	Cell membrane disruption	(Hao et al. 2024)
cLFchimera	Camel milk lactoferrin	α-helixes (chimeric peptide)	Avian bacterial pathogens - *Staphylococcus aureus* - *Streptococcus epidermidis* - *Pseudomonas aeruginosa* - *Escherichia coli* - *Salmonella enteritidis* - *Klebsiella pneumoniae*	- Membrane disruption/permeabilization - Intracellular effects	(Tanhaiean et al. 2018)
Fowl-1 (8–26)-WRK	Chicken (derived from fowlicidin- 1)	α-helical	Gram-negative and gram-positive bacteria Antibiotic-resistant strains - MRSA - MDRPA - VREF	Membrane disruption/permeabilization	(Rajasekaran et al. 2019)
Metchnikowin II (MetII)	Drosophila	Proline-rich	Avian *Pasteurella multocida*	Enhances immune responses (adjuvant-like effect)	(MINGFU et al. 2023)
Liver-expressed antimicrobial peptide 2 (LEAP-2)	Duck (*Anas platyrhynchos domesticus*)	Cysteine-rich	- Gram-positive bacteria: *Listeria monocytogenes*, *Staphylococcus aureus* - Gram-negative bacteria: *Escherichia coli*, *Salmonella enterica* serovars (Choleraesuis, Typhimurium, Enteritidis)	Cell membrane disruption	(Hong et al. 2019)
Platelet-derived AMPs - HPAP - CaPAP - ChPAP	- Horse platelets - Cattle platelets - Chicken platelets	Not specified	- *S. aureus* - E. *coli*	Cell membrane disruption	(Vasilchenko et al. 2015)
ChBac3.4	Domestic Goat (*Capra hircus*)	Proline-rich	- Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*) - Gram-positive bacteria (*S. aureus*)	- Membrane disruption/permeabilization - Protein synthesis inhibition	(Kopeikin et al. 2020)

Table 6 (continued)

AMP Name	Source	Structural Class	Target Pathogen/Activity	Mechanism of Action	Source
Pm11	Pleurocidin of *Pleuronectes americanus* (winter flounder)	Cationic α-helical	Bovine mastitis pathogens - *Escherichia coli* - *Staphylococcus aureus* - *Streptococcus agalactiae* - *Streptococcus uberis* - *Klebsiella* spp.	- Cell membrane disruption - Induces morphological changes	(Popitool et al. 2022)
- Melittin - Secapin-1	*Apis mellifera* (honey bee)	Not specified	Capripoxvirus (SPPV, GTPV, LSDV)	Binds to DNA-directed RNA polymerase	(Mustafa et al. 2023)
Aureocin A53	*Staphylococcus aureus* A53 from pasteurized milk	N-formylated	- *Staphylococcus aureus* - *Streptococcus agalactiae* - (both are related to bovine mastitis)	Cell membrane disruption	(Marques-Bastos et al. 2022)
Microplusin	Cattle tick *Rhipicephalus (Boophilus) microplus*	α-helical globular domain	- Gram-positive bacteria (e.g. *Micrococcus luteus*) - Fungi (e.g. *Cryptococcus neoformans*)	Copper ion sequestration	(Silva et al. 2011)
Ixodidin		Cysteine-rich peptide	- *Micrococcus luteus* (gram-positive) - *Escherichia coli* (gram-negative, less susceptible)	Proteinase inhibitory activities	(Fogaça et al. 2006)
Ctx (Ile21)-Ha	Brazilian frog (*Hypsiboas albopunctatus*)	α-helical and cationic peptide	*Salmonella typhimurium, Salmonella enteritidis, Pseudomonas aeruginosa* (MDR), *Staphylococcus aureus*, (MDR), *Acinetobacter baumannii* (MDR)	Cell membrane disruption (barrel-stave model)	(Roque-Borda et al. 2021a, b; Roque-Borda et al. 2021a, b)
No specific name - KGGDLGLFEPTL - FELPLGAG - CASALLGA	Chicken egg yolk	Random coil	*S. aureus*, *B. cereus*, S. *typhimurium*	Cell membrane disruption	(Pimchan et al. 2023)
Gal-13	Chicken	Cationic β-defensins 13	E. *coli*, *Enterococcus* sp.	Cell membrane disruption	(Y. Wang et al. 2023)
β-defensins1	Chicken	Cationic β-defensins 1	*Eimeria* sp. (coccidia)	Cell membrane disruption	(Mahmoud et al. 2023)
A11	Modified from acidocin J1132β (*Lactobacillus acidophilus* JCM 1132)	α-helical	*Salmonella enterica* serovar *Typhimurium*	- Membrane disruption/permeabilization - Intracellular effects (DNA binding)	(Sengkhui et al. 2023)
- P1 (NPSRQERR) - P2 (PDENK) - P3 (VHTAPK)	*Lactobacillus rhamnosus*	-	Avian pathogenic *Escherichia coli* (APEC)	Cell membrane disruption affecting MlaA-OmpC/F system	(Kathayat et al. 2021)
RSRP	rabbit sacculus rotundus-derived	-	Very virulent infectious bursal disease virus (vvIBDV)	Reduced apoptosis and mast cell activation	(D. Wang et al. 2024)

Table 6 (continued)

AMP Name	Source	Structural Class	Target Pathogen/Activity	Mechanism of Action	Source
OaBac5 mini	Sheep neutrophils	Linear proline-rich antimicrobial peptide (PrAMP)	*Salmonella enterica* serovar Pullorum (S. *pullorum*)	Modulates innate immunity through the TLR4/MyD88/NF-κB pathway	(Shen et al. 2023)
ABP	Chicken feathers	α-helix (64.30%) and β-sheet (33.40%)	Methicillin-resistant *staphylococcus aureus* (MRSA)	Cell membrane disruption	(Alahyaribeik & Nazarpour 2024)

Abbreviations: *AMP* antimicrobial peptide, *BHV- 1* bovine herpesvirus type 1, *SARS-CoV- 2* severe acute respiratory syndrome coronavirus 2, *HSV- 2* herpes simplex virus 2, *DENV- 2* dengue flavivirus 2, *CHIKV* chikungunya virus, *ASFV* african swine fever virus, *MDR* multidrug resistance, *MRSA* methicillin-resistant *S. aureus*, *MDRPA* multidrug resistant *Pseudomonas aeruginosa*, *VREF* vancomycin-resistant *Enterococcus faecium, SSPV* sheep pox virus, *GTPV* goat pox virus, *LSDV* lumpy skin disease virus, *DNA* deoxyribonucleic acid, *RNA* ribonucleic acid

With the emergence of new animal pathogens, including zoonotic diseases that pose a risk to both animal and human health, and the evolution of existing pathogens, peptide-based strategies that utilize the flexibility and specificity of peptides to develop innovative preventive and therapeutic approaches for animal diseases deserve more extensive investigation to ultimately address emerging threats.

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Authors' contributions
A.C.P.: investigation, resources, visualization, writing-original draft. Y.X.D.: resources, Writing—review & editing. X.J.W.: resources, writing—review & editing. H.F.: conceptualization, supervision, validation, project administration.

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Consent for publication
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