Received: 30 September 2022 | Revised: 30 November 2022 | Accepted: 1 December 2022

DOI: 10.1002/psc.3466

REVIEW

Journal of PeptideScience WILEY

Artificial peptides to induce membrane denaturation and disruption and modulate membrane composition and fusion

Ilze Lāce[1] | Elena R. Cotroneo[1] | Nils Hesselbarth[1] | Nadja A. Simeth[1,2]

[1]Institute for Organic and Biomolecular Chemistry, Department of Chemistry, University of Göttingen, Göttingen, Germany

[2]Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Göttingen, Germany

Correspondence
Nadja A. Simeth, Institute for Organic and Biomolecular Chemistry, Department of Chemistry, University of Göttingen, Tammannstr. 2, 37077 Göttingen, Germany.
Email: nadja.simeth@uni-goettingen.de.

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: EXC 2067/1-390729940

Membranes consisting of phospholipid bilayers are an essential constituent of eukaryotic cells and their compartments. The alteration of their composition, structure, and morphology plays an important role in modulating physiological processes, such as transport of molecules, cell migration, or signaling, but it can also lead to lethal effects. The three main classes of membrane-active peptides that are responsible for inducing such alterations are cell-penetrating peptides (CPPs), antimicrobial peptides (AMPs), and fusion peptides (FPs). These peptides are able to interact with lipid bilayers in highly specific and tightly regulated manners. They can either penetrate the membrane, inducing nondestructive, transient alterations, or disrupt, permeabilize, or translocate through it, or induce membrane fusion by generating attractive forces between two bilayers. Because of these properties, membrane-active peptides have attracted the attention of the pharmaceutical industry, and naturally occurring bioactive structures have been used as a platform for synthetic modification and the development of artificial analogs with optimized therapeutic properties to transport biologically active cargos or serve as novel antimicrobial agents. In this review, we focus on synthetic membrane interacting peptides with bioactivity comparable with their natural counterparts and describe their mechanism of action.

KEYWORDS
antimicrobial peptides, cell penetrating peptides, membrane, peptides, SNAREs

1 | INTRODUCTION

Lipid bilayers are key structural components of both prokaryotic and eukaryotic cells. They not only represent the barrier separating cells from the outside environment but also determine the internal compartmentalization of the cell itself. In eukaryotic cells, membranes of organelles allow, for instance, protection of special cargos, such as DNA, buildup of electrochemical gradients, and drive of endergonic reactions, as well as separation of reactive intermediates from the surrounding environment. Moreover, the membrane serves as an anchoring moiety for the cytoskeleton building the structure of the cell and accommodates cellular receptors, channels, and transporters to facilitate communication and cargo exchange.

Although the stability, integrity, and composition of lipid bilayers are substantial to maintain these functions, their alteration can lead to perturbations of cellular processes or content leakage in both prokaryotic and eukaryotic cells. On the other hand, the membrane composition is naturally changed to facilitate vesicular transport processes, such as neurotransmitter release. Oftentimes, peptides capable of specifically interacting with cellular membranes are key players in

Ilze Lāce, Elena R. Cotroneo, and Nils Hesselbarth contributed equally.

these alterations.[1] Prominent examples are fusion peptides, such as soluble *N*-ethylmaleimide-sensitive factor attachment proteins (SNAREs), antimicrobial peptides (AMPs), and cell-penetrating peptides (CPPs; Figure 1).

CPPs are small, linear peptides able to cross the lipid bilayer while transporting biologically active cargos. This property makes CPPs highly attractive targets for the development of artificial analogs as tools for drug delivery. A vast library of natural compounds is already available and continuously growing, with new CPPs being discovered.[2,3] Because of low activity or high toxicity of the natural compounds, though, new, synthetic molecules with similar abilities but improved characteristics are getting developed.[4,5] Despite the vast number of studies on the different internalization modes of CPPs, not all parameters influencing their behavior are yet fully understood. Thus, the design of unnatural CPPs is key to rationally investigate structure–activity relationships. A large number of nonnatural compounds have thus been synthesized, such as the chimeric transportan[6] and his numerous derivatives, a library of polyarginine peptides,[7] and many more. The use of unnatural amino acids, the combination with lipid chains, or the fusion of different domains with cell-penetrating abilities are strategies that have been employed to generate new and interesting compounds with higher activity and improved characteristics regarding, for example, their toxicity, compared with their natural analogs.

In contrast, AMPs are involved in the innate immune system of multiple organisms, by acting against bacteria, fungi, and viruses.[8] Because of their structural properties, they are capable of interacting in a unique way with lipid bilayers. This allows them to disrupt, permeabilize, or translocate through both eukaryotic and prokaryotic cell membranes. Their destructive interactions with pathogenic cells became interesting for pharmaceutical industry as they bear the potential of replacing classical antibiotics. Thus, artificial derivatives with optimized properties are highly desired in both basic and applied research.[9,10] Different modifications have been carried out on natural AMPs to generate more effective and potent analogs. Alteration of the net charge,[11] secondary structure,[12] or stereochemistry[13] of natural AMPs are just some of the modifications carried out in order to generate a higher antimicrobial activity than the one expressed by natural compounds.

Another important class of proteins that interact with the phospholipid membrane is fusion peptides. These proteins can initiate and regulate membrane fusion, which is the event where two different lipid bilayers merge into one.[14] In eukaryotic organisms, membrane fusion is involved in several vital processes including neurotransmitter release, gamete fusion, placenta formation, ocular lens formation, and muscle differentiation and is regulated by fusion proteins.[15,16] The most prominent ones are SNARE proteins, which are predominantly involved in vesicle fusion, including exocytosis as well as fusion of vesicles with membrane-bound organelles.[17,18] Several synthetic analogs of these peptides have been developed that can mimic the function of fusion peptides by utilizing more easily modifiable structures.

Besides these three classes, a variety of other membrane-active peptides with different abilities and structures exist. Peptides that can alter membrane morphology, geometry, and composition have been discovered and synthesized.[19,20] Furthermore, fibrillogenic peptides and protein-interacting peptides that exert their activity on the plasma membrane are also being studied Ultimately, structural analogs such as β-peptides or cyclic peptides have also gained attention as possible targets for drug development, especially because of their resistance against enzymatic metabolization.

Because of their versatile operating principles, membrane-interacting peptides have been inspiring researchers to derive artificial peptide and peptide-hybrid constructs harvesting the properties of their natural analogs. In this manner, novel biologically active molecules were developed to potentially serve as next-generation pharmaceuticals and as tool compounds for the in-depth study of membrane composition, stability, and fusion. In this review, we will discuss the different artificial peptide models that have been designed to mimic the function of cell-penetrating, antimicrobial, and fusion peptides of natural origin.

2 | CELL-PENETRATING PEPTIDES

CPPs are mainly small and cationic peptides consisting of 5–30 amino acids that are able to move through the lipid bilayer. Because of their ability to carry biologically active cargos, such as proteins,[21,22] nucleic

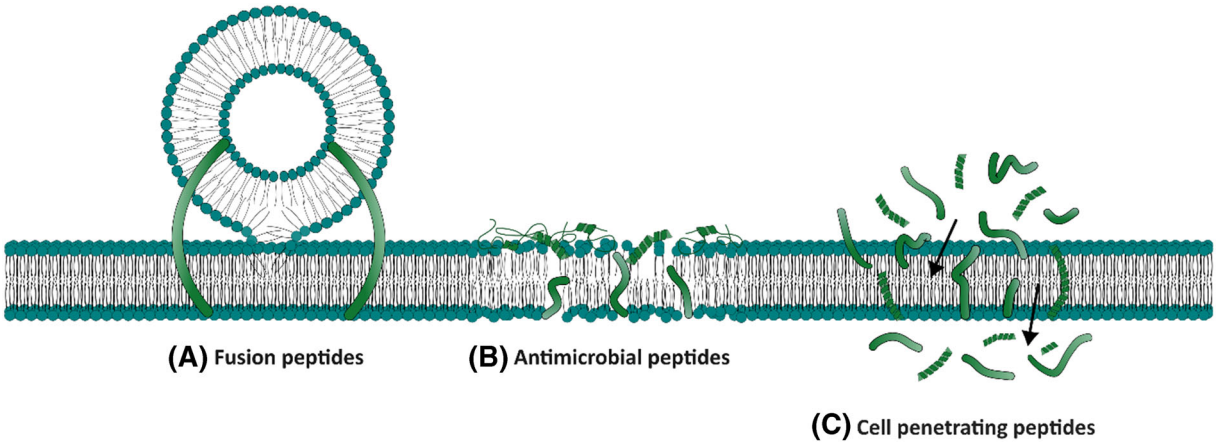


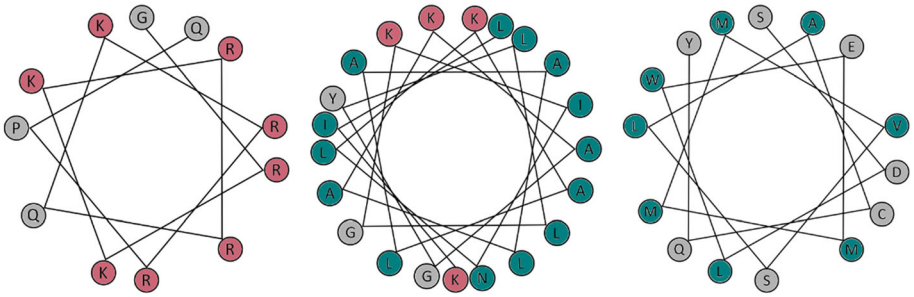
FIGURE 1 Representation of membrane interaction of (a) fusion peptides, (b) antimicrobial peptides, and (c) cell-penetrating peptides

acids,[23] or nanoparticles[24,25] across the membrane, they are also referred to as protein transduction domains (PTDs). The first CPPs to be discovered were the HIV-1 TAT protein in 1988[26] and penetratin in 1994.[27] These natural peptides captured the interest of scientists because of their ability to transport hydrophilic compounds through the plasma membrane, in a non-cell-specific but highly efficient manner. The possibility to exploit this characteristic for therapeutic approaches entailed a tremendous growth of the field, with many more CPPs being discovered and researchers developing new model peptides with similar behaviors. Moreover, CPPs have gained a significant relevance in the biomedical and pharmaceutical fields because of their extremely low cytotoxicity and have been employed either for cellular delivery of molecules with therapeutic activity or as compounds able to target intracellular structures such as the cytoskeleton or chromosomes.[23,28] Although CPPs able to cross prokaryotic membranes are known,[29,30] the vast majority of compounds belonging to this class is used to vehiculate cargos into eukaryotic cells.

CPPs can be divided into different classes according to their chemical structure, namely, hydrophobic, cationic, amphiphilic, and membranotropic CPPs (see Figure 2). Hydrophobic CPPs are a small class of compounds that exhibit an abundance of nonpolar amino acids—such as alanine, phenylalanine, tryptophan, methionine, and tyrosine—in their backbone, resulting in a low net charge (Figure 2). Hydrophobic CPPs seem to be able to spontaneously penetrate the membrane in a fully energy-independent mode, which makes them an interesting class to study. Representatives of natural origin are not vastly investigated,[31,32] but chemically modified hydrophobic CPPs have been synthesized and include stapled peptides,[33] prenylated peptides,[34] and pepducins.[35]

In contrast, cationic CPPs are mainly arginine-,[36] histidine-,[37] or lysine-rich[38] peptides (Figure 2). Within these, histidine-rich CPPs are a specialized class of compounds and are mainly used for the transport of siRNA or DNA derivatives inside cells.[39] Cationic CPPs include natural exponents, such as the aforementioned TAT and penetratin, as well as a large number of oligoarginines, that can be both of natural and synthetic origin. Specifically, polyarginines are an interesting class of compounds and have been thoroughly investigated because of their highly efficient internalization and ability to deliver bioactive molecules. Studies on polyarginine peptides of different lengths (R3 to R12) were carried out in order to establish the optimal chain length for internalization. Confocal microscopy analysis on live cells showed that in order for arginine-rich peptides to be able to cross the plasma membrane, they require at least six arginine residues, and increasing this number promotes the internalization efficiency.[40] Once the minimal structure required for penetration was identified, arginine sequences were then employed for the internalization in live cells of various cargos, such as peptides, oligonucleotides, or nanoparticles (vide infra).[41–43]

Amphiphilic CPPs are composed of both polar and nonpolar amino acids (Figure 2). The resulting charge of the peptide can thus be positive, neutral, or negative. Transportan is the primary example for this class of CPPs. It is a synthetic 27-residue chimeric CPP in which



Cationic	Sequence	Origin
TAT	GRKKRRQRRRPQ	HIV Tat protein
Penetratin	RQIKIWFQNRRMKWKK	Antennapedia homeodomain
Polyarginines	R_n (with n = 6 – 12)	Synthetic
Amphiphilic		
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL	Galanin and mastoparan
Transportan-10	AGYLLGKINLKALAALAKKIL	Transportan
PepFect 14	Stearyl-AGYLLGKLLOOLAAAALOOLL	Transportan
NickFet 55	(Stearyl-AGYLLG)δ-OINLKALAALAKAIL	Transportan
Membranotropic		
gH625	HGLASTLTRWAHYNALIRAF	HSV 1
Hydrophobic		
Pep7	SDLWEMMMVSLACQY	CHL8 peptide

FIGURE 2 Helical diagrams displaying the sequence of exemplary cationic, amphiphilic, membranotropic, and hydrophobic CPPs, with cationic amino acids in pink, hydrophobic amino acids in blue, and non-hydrophobic and non-cationic residues in grey. The table shows main examples of the CPP classes including their amino acid sequence and their natural or synthetic origin, respectively.

the first 12 residues derive from the neuropeptide galanin and are then linked through a lysine to 14 residues derived from the peptide toxin mastoparan.[6] The newly formed peptide was named transportan because of its ability to deliver cargo molecules, which were covalently connected to the Lys[10] residue, into the cell. Transportan has been proven to carry small cargos through the membrane at low concentrations and low temperatures (4°C). This is particularly important because it shows that the penetration mechanism of transportan does not require a high cellular energy expenditure, but it is mostly a passive process. However, at high concentrations, the peptide shows cytotoxicity because of pore formation and subsequent permeation. Different modifications were introduced on the original molecule, in order to simplify the structure while improving the activity and reducing the cytotoxic effects.[44] The first derivative of transportan was transportan-10 (TP10; Figure 2), a shortened version of the original peptide with higher cellular translocation activity and lower toxicity.[45] Following TP10, a large number of derivatives and structural analogs were synthesized, which generated two new CPP series: PepFects[46] and NickFects[47] (Figure 2). Both series are derived from the stearylation of transportan, leading to a higher degree of cargo delivery into the cell. Subsequently, different modifications were carried out, such as substitution of lysine residues with nonnatural ornithines, or the introduction of a phosphoryl group in the peptidic backbone.[48] All these alterations aimed to improve the general activity of transportan. PepFects and NickFects indeed exhibit overall increased transport activity and lower toxicity compared with parent transportan, making them interesting new compounds for the vehiculation of biologically active molecules into eukaryotic cells. These characteristics are mainly due to non-covalent interactions with their cargos, which induce nanoparticle formation. This delivery system allows for an easier release of the active molecules once the target site is reached, in contrast to covalent-bound cargos.

Membranotropic CPPs are a class of compounds that also exhibit amphiphilic characteristics. Nevertheless, the primary attribute that distinguishes peptides belonging to this class is the presence of both large aromatic residues and small residues in their sequence. In general, membranotropic CPPs have a high abundance of alanine and glycine residues in their backbone, which allow flexibility in the strands. On the other hand, the hydrophobic, aromatic residues determine a favorable interaction with the membrane. The primary exponent of this class of compounds is the gH625 peptide, derived from the glycoprotein H of the herpes simplex virus type I.[49] The peptide is constituted of a sequence of 20 residues and has a membrane-perturbing domain rich in hydrophobic amino acids including alanine, glycine, and leucine, as well as aromatic residues such as tyrosine and tryphtopan.[50]

In order for CPPs to exert their primary activity in vivo, they first need to reach their site of action on the plasma membrane. Interactions between CPPs and serum proteins can affect their ability to penetrate the lipid bilayer and thus carry cargos into cells.[51,52] Specifically, studies have been carried out on the influence of serum proteins on the internalization of arginine-rich CPPs, showing that the cellular uptake of peptides such as R4, R8, R12, and R16 is significantly lowered in serum compared with aqueous media without serum proteins. The longer peptides, R12 and R16, have shown the most significant decrease in internalization, which has been linked with their higher binding affinity with serum proteins.[53] Despite the negative impact that serum can have on their availability, CPPs have been shown to also benefit from the binding to serum proteins by elongating their circulation half-life.[54]

Once they have reached the plasma membrane, CPPs are crossing the lipid bilayer to transport their bioactive cargos to their cellular targets. The mechanism of internalization they follow differs strongly between various representatives and is independent from the structural motifs they are based on. Thus, one of the most crucial aspects in the design and development of CPPs is to understand and study the mode of action of these compounds. Up to now, several internalization modes have been identified; however, it is still unclear which parameters in CPPs determine which mode they will access. Although CPPs are usually classified by their structural characteristics, the different classes are not linked to a class-specific penetration mechanism. On the contrary, structurally similar CPPs sometimes follow significantly different penetrating pathways. Generally, the behavior of CPPs appears to depend on various factors, including both intrinsic characteristics of the peptide and experimental parameters, such as peptide concentration or targeted cell type. For these reasons, the same molecule can undergo different internalization processes, under different conditions. The two most common ways of internalization for CPPs are direct penetration[55] and endocytosis[56] (Figure 3). In the following sections, we will discuss the three different internalization pathways using suitable examples from various classes of CPPs. Eventually, a deeper understanding of these mechanisms could lead to the development of useful tools to control the function of CPPs by design.

2.1 | Direct penetration

Direct penetration is one of the main mechanisms that CPPs use to cross the plasma membrane. This pathway is especially undertaken by positively charged CPPs. These molecules interact with the negatively charged cell surface, causing membrane destabilization and alteration and, ultimately, entry of the CPP. This process of internalization is an energy-independent one; therefore, it does not require the system to spend energy to promote it.

Direct penetration of CPPs is known to be correlated with membrane potential.[57,58] The charge imbalance between the inside and outside of the cell membrane can promote internalization of CPPs. The membrane potential can be additionally altered by cationic CPPs adsorbing on the membrane. This can eventually induce pore formation and thus increase CPP penetration.[59] Internalization through direct penetration can follow different pathways, such as pore formation,[60] the carpet-like model,[61] and transduction via inverted micelles (Figure 4).[62] The mechanisms that CPPs use for direct penetration are correlated with the ones that AMPs use to denature the plasma membrane (vide infra). The main difference between these

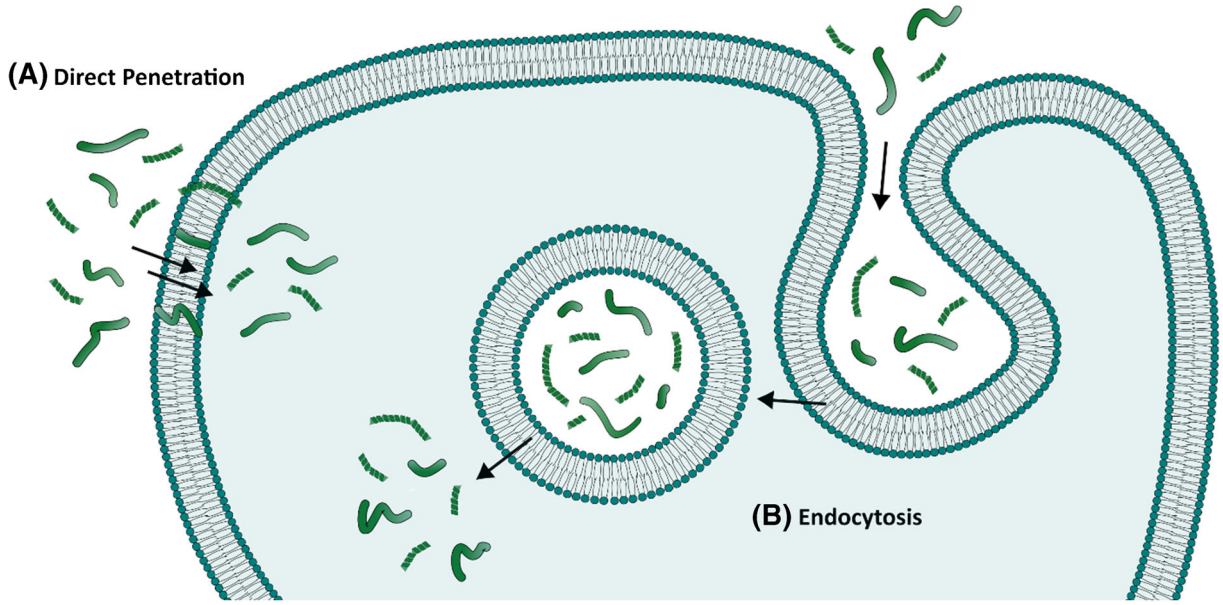


FIGURE 3 Representation of (a) direct penetration and (b) endocytosis mechanisms for CPPs internalization

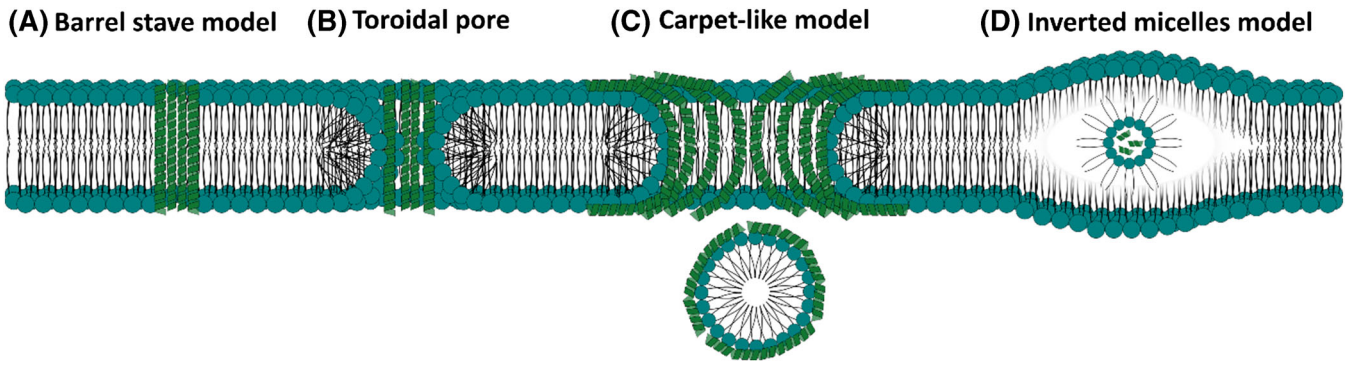


FIGURE 4 Different direct penetration pathways. (a) Barrel stave model—pore formation delimited by the peptidic strands of CPPs; (b) toroidal pore model—formation of a pore delimited by peptide strands and phospholipids; (c) carpet-like model—a locally high concentration of peptides induces destabilization of the membrane, formation of a transient hole, and subsequent formation of a micelle that delivers the CPPs inside the cell; (d) inverted micelles model—the reorganization of the membrane leads to the formation of a micelle in which the polar heads of the phospholipids are on the inside, surrounding the peptides.

two scenarios—penetration versus denaturation—is the concentration of the peptides to which the membrane is exposed to. For CPPs, it is very important to modulate the concentration to prevent toxic effects. Another strong difference between CPPs and AMPs is the type of membranes they are employed to interact with. Whereas CPPs are mainly studied for penetration of eukaryotic cells, AMPs are useful tools for the denaturation of prokaryotic cell membranes.

Pore formation is a well-described mechanism of internalization exhibited by a vast number of CPPs from different classes. Pep-1, for instance, is a 21-residue chimeric peptide that can cross the plasma membrane through formation of pore-like structures.[63] Generally, the mechanism of pore formation can proceed by following either the barrel stave model or the toroidal pore model. Both pathways are observed in amphipathic CPPs. In the first system, CPPs initially reach the membrane surface as monomers or oligomers. They then start assembling to form bigger complexes. Once the full bundle is formed, it inserts in the lipid bilayer and increases in size because of the recruitment of additional monomers. An example of a natural CPP that follows this pattern is alamethicin,[64] which forms a barrel stave pore with eight helices (Figure 4). In the final state of the pore, the peptides are aligned so that the hydrophobic residues are facing toward the lipid chains of the membrane, whereas the hydrophilic residues are lining along the internal pore forming a hydrophilic pocket. In the toroidal pore model, the CPPs initially reach the membrane surface and orient themselves parallel to it, in an inactive state. When the local ratio between peptide and lipid concentration is high enough, the pore is formed. In contrast to the barrel state model, both peptides and lipids rearrange, so that the positively charged heads of the phospholipids and the peptidic chains of the CPPs are actively lining the borders of the pore. Toroidal pores are generally larger in diameter

compared with the barrel stave ones because of the active participation of membrane lipids in the structure of the pores, and they have been observed more often.[65,66]

The carpet-like model is an internalization process mainly known for AMPs (vide infra).[67] This pathway is also used by CPPs, although it might result in cytotoxic effects at high peptide concentrations, because of breakage of the membrane. When following this internalization pathway, CPPs initially lie parallelly aligned on the membrane surface. The carpeting effect, determined by the peptides covering the lipid bilayer, is induced by an increase in local concentration of the peptide. Once the threshold concentration is reached, the active internalization process starts.[68] Rotation of the peptides toward the inside of the membrane leads to a destabilization and reorganization of the latter, with the subsequent formation of a micelle and of a transient hole allowing the peptides to cross the membrane (Figure 4).[61] Although the mechanism is similar to the one of pore formation, there are a few key differences. In the carpet-like model, the membrane-bound peptide monomers do not need to co-assemble into a complex macromolecular structure to start the internalization. The process is also less structure-specific, so different CPPs are able to penetrate through the plasma membrane via this pathway.

Inverted micelles are another internalization strategy utilized by CPPs to penetrate inside the cell.[69] The proposed mechanism for this process suggests that in the first step, the positively charged residues of the peptide interact with the negatively charged membrane. Subsequently, CPPs, especially such that exhibit hydrophobic amino acids in their sequence, will interact with the lipid bilayer and facilitate a structural re-organization of the latter: After the membrane curvature is altered, an invagination of the membrane occurs and inverted micelles are formed within the lipid bilayer (Figure 4).[70] The micelles then reach the cytosol and are destabilized because of the change of environment leading to release of the CPPs and the cargo they were loaded with. Despite the lack of hydrophobic residues in their structures, also CPPs such as TAT peptide and octaarginine can follow this mechanism of internalization.[71]

Not all CPPs selectively follow just a single pathway to cross the cell membrane. For instance, the internalization process of arginine-rich CPPs is still under debate. Initially, it was hypothesized that they cross exclusively via direct penetration. This has been proven to be an artifact caused by fixation of cells with methanol during analysis, which led to membrane disruption.[72] More recent studies have shown that arginine-rich CPPs mainly undergo internalization through endocytosis (vide infra). Although endocytosis appears to be the main penetration pathway, the presence of arginine-rich peptides in the cytosol and the nucleus has still been observed in cells at 4°C. These findings, correlated with the diffused distribution inside the cell, point to the capability of arginine-rich peptides to also pass through the plasma membrane via direct penetration.[53] Interestingly, the equilibrium between the two pathways is shifted depending on various parameters, including the type of peptide and its concentration,[73] size and features of the cargo,[74] and membrane composition.[75] Moreover, it was also shown that the direct internalization of arginine-rich peptides is promoted by co-administering them with adjuvating molecules, such as peptides that are able to alter the density of the lipid phase of the plasma membrane.[76]

Another important aspect affecting the internalization pathway, especially regarding transportan and its derivatives and arginine-rich CPPs, is the lipid phase composition of the cell membrane, and in particular the amount of cholesterol.[77,78] In the case of transportan, it has been observed that high levels of cholesterol lead to a greater degree of rigidity of the membrane and thus inhibit the ability of the peptide to cross the lipid bilayer.[79] On the other hand, studies have proven that sequestering cholesterol from the membrane can promote the internalization of arginine-rich peptides via endocytosis (vide infra) while inhibiting their direct penetration.[73]

Besides the three aforementioned types of direct penetration, other entry pathways have been observed in synthetic models. For instance, KYp is a synthetically modified anticancer peptide that is able to permeate through the plasma membrane because of a unique mechanism. The phosphorylation of the peptide strand determines its interaction with the membrane-abundant alkalyne phosphatase (ALP). The dephosphorylation of KYp by the enzyme induces self-assembly of the peptidic strands, which subsequently determines phase change and therefore membrane leakage and internalization of the peptide.[80]

2.2 | Endocytosis

2.2.1 | Endocytosis pathways

Endocytosis is an energy-dependent mechanism that represents the most common pathway of internalization for a wide range of macromolecules. These are transported from the extracellular space to the cytosol in vesicles or vacuoles, which are formed from invaginations of the membrane.[81] Once the vesicles or vacuoles have reached the cytosol, the molecules carried inside need to be released via endosomal escape (vide infra) in order to reach their intracellular targets. The endocytic process can be divided into two main categories, namely, phagocytosis and pinocytosis. The first one refers to the transport of large molecules and is limited to specialized cells, whereas the second one involves the transport of fluids and small solutes and is performed by all cells. Pinocytosis can occur following different mechanisms, including macropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, and clathrin- and caveolae-independent endocytosis (Figure 5). Which pathway the CPPs follow depends on intrinsic characteristics of both the peptides and the cells.[82–84] In the following paragraphs, we will highlight selected illustrative examples of each one of them.

Macropinocytosis is a rapid process that occurs at membrane sites rich in lipid rafts. Although numerous enzymes can assist in the process, macropinocytosis mainly depends on the interaction of actin and the plasma membrane, which results in the formation of lipid rafts.[85] Many CPPs, such as TAT[86] and short arginine-rich peptides,[84,87] are internalized via this mechanism. Studies have shown that this internalization pathway is induced by the peptides interacting with specific parts of the membrane on the outside of eukaryotic cells

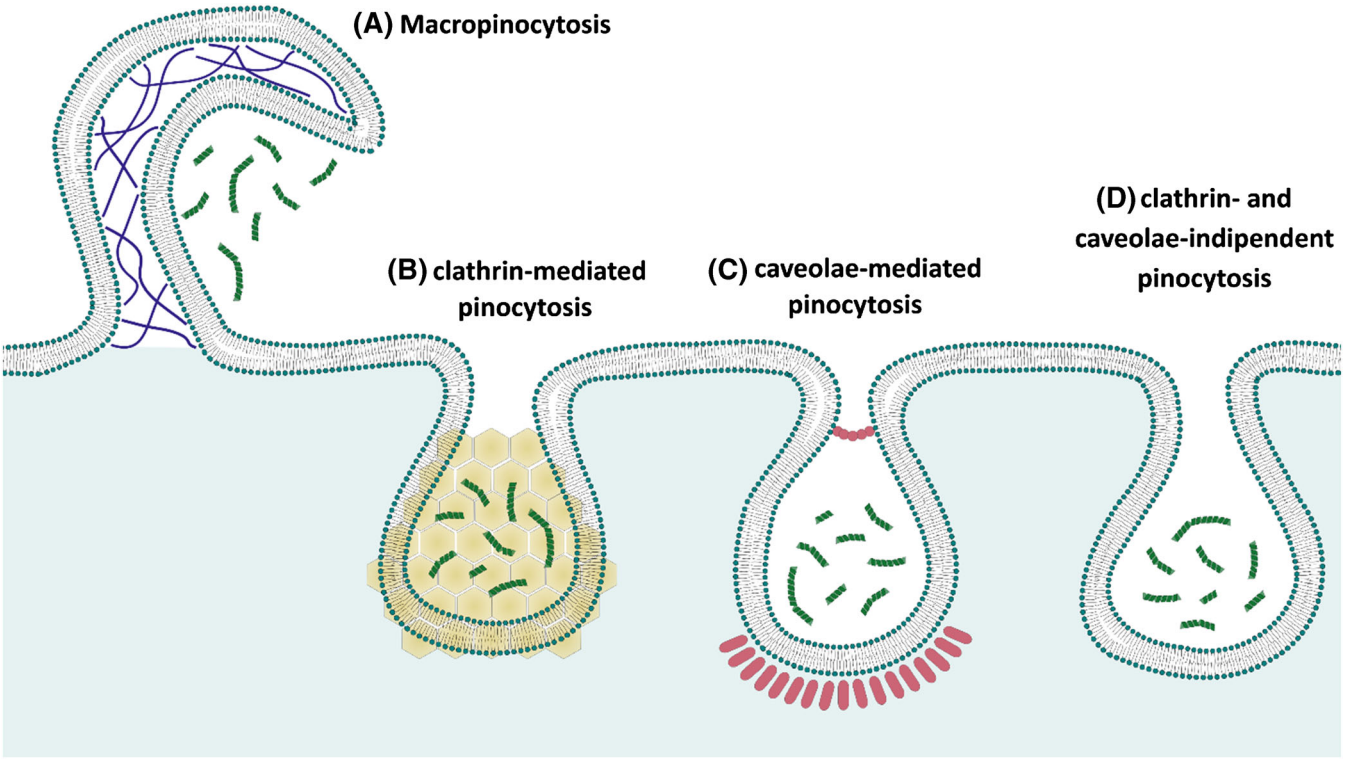


FIGURE 5 Different pinocytosis pathways. (a) Macropinocytosis; (b) clathrin-mediated endocytosis; (c) caveolae-mediated endocytosis; (d) clathrin- and caveolae-independent endocytosis

and eventually transmitting a signal to the actin filaments of the cytoskeleton on the inside face of the plasma membrane. Tubulating protrusions are then formed by the actin filaments on the outside face of the membrane, which then collapse back on the membrane surface forming endocytic vesicles called macropinosomes (see Figure 5). Many short arginine-rich CPPs have been shown to be internalized via macropinocytosis. The uptake of the oligoarginine, for instance, is correlated to a rearrangement of actin filaments in the cytoskeleton, and its cellular internalization can be suppressed by macropinocytosis inhibitors.[88] The internalization of R12, on the other hand, is directly correlated to the presence of CXCR4, a chemokine receptor able to promote macropinocytosis.[87] Not only oligoarginine-based CPPs use macropinocytosis as an internalization pathway but also other classes of CPPs such as transportan derivatives. NF1, a synthetic anionic CPP belonging to the NickFect family, showed uptake via macropinocytosis as its only transfection pathway. NF51, another representant of the same family, is able to penetrate the plasma membrane through macropinocytosis as well as through other endocytic pathways.[89]

An additional pathway that CPPs use to penetrate through the plasma membrane is clathrin-mediated endocytosis. Clathrin is a membrane protein involved in the coating and shaping of spherical vesicles responsible for intracellular transport. This tightly regulated and complex endocytic mechanism is well characterized and accessible to a wide range of CPPs, including oligoarginines, TAT peptides, and anionic CPPs. The clathrin-mediated endocytosis is initiated by a ligand interacting with a specific membrane receptor. Subsequently, clathrins are recruited in that area of the membrane, followed by the formation of the coated endocytic vesicles and their loading with the extracellular cargo. Once the vesicles are formed, they move toward the inside of the cell where they fuse with endosomes, terminating the process (Figure 5). Clathrins are crucially involved in every step and are ultimately recycled once the vesicles fuse with the endosomes. A CPP following clathrin-mediated endocytosis is, for instance, R8.[90] The process is mediated through the interaction of the oligoarginine strand with the syndecan-4 receptor, which is also involved in macropinocytotic processes.[84] As per anionic CPPs that can follow clathrin-mediated endocytosis, NF51 from the NickFect family of transportan derivatives can be transfected inside cells through this pathway, among others.[89]

Caveolae are large invaginations on the membrane surface, rich in cholesterol and sphingolipids. For this reason, they have also been called lipid rafts. This local concentration of lipids in specific areas of the membrane is induced by caveolin proteins, which confer the shape and structure of the caveolae. This endocytic process appears to be directly connected to the activity of actin filaments in the cytoskeleton (Figure 5). Different studies suggest that the caveolae-mediated internalization of CPPs is determined by the size and nature of the attached cargos. For instance, TAT peptide has been shown to undergo caveolae-dependent endocytosis, but only when it is fused with larger cargos such as proteins.[91] Similarly, transportan and TP10 uptake has been shown to follow caveolae-mediated endocytosis, when the CPPs are linked to cargos of considerable size.[92] Also, PepFect14, a

synthetic anionic derivative of transportan, has been shown to be internalized through the same endocytic pathway. This seems to be consistent with the notion that the major determinant for CPPs to follow this internalization mechanism is the size of the cargo.[93]

The fourth known mechanism for pinocytosis, clathrin- and caveolae-independent endocytosis (Figure 5), is an internalization method that, for CPPs, has been rarely observed. Two examples are the internalizations of transportan and TP10. These two CPPs can undergo uptake through caveosomes, but another internalization process possibly involving flotillins has also been postulated.[92] The most recent example of a CPP being internalized through pinocytosis is LMWP, a synthetic CPP covalently conjugated to siRNA that mediates its transport into cells.[94]

2.2.2 | Endosomal escape

The final step of the endocytic process, independent from the mechanism the CPPs are using to enter the cell, is the endosomal escape.[95] Once CPPs have passed the plasma membrane, they tend to accumulate in so-called endosomes. There, they could be trapped and subsequently metabolized, without reaching the cytosol, in which their cargos would exert their biological functions.[96] Proton sponge effect,[97] pore formation,[98] membrane disruption,[99] and membrane budding[100] are the mechanisms that can determine endosomal escape of CPPs, among others (Figure 6).

As endosomal escape appears to be the limiting step for the internalization of CPPs via endocytosis, several systems have been tested to facilitate this process. The endosomal lumen has a significantly lower pH than the cytosol (pH 5 vs. ~pH 7). Therefore, multiple strategies that exploit this specific characteristic have been developed. One example is a histidine-tagged variant of listeriolysin O, which was modified at position 311 by substitution of a histidine with an alanine residue. This modification results in a pH-dependent threshold for the pore formation activity of this peptide so that pore formation can only take place at pH < 6.[98] Pores are therefore only formed in the endosomal membrane and not yet in the plasma membrane. Another example of pH-dependent activity to induce endosomal escape is the rational design of Glu-based membrane lytic CPPs derived from the natural ponericin W3 and melittin peptides.[101] In this case, the introduction of glutamic acid residues determines a decrease in hydrophilicity of the peptides at low pH and therefore manifestation of lytic activity toward the endosomal membrane. A similar strategy had been already tested, with a modification of the natural venom peptide Mastoparan X. This time, the strand was modified both by substitution of a methionine residue with a norleucine and with the addition to the N-terminus of three Glu residues. This determined selective membrane lytic activity toward endosomal membranes and not cellular ones.[102]

All the aforementioned endocytic processes can easily determine the internalization of larger cargos, such as proteins or siRNAs. The appealing possibility of vehiculating big biomolecules inside the cell opened up a vast research field on synthetic peptides that could either undergo endocytosis themselves, promote the endocytic process for other peptides,[103] or induce endosomal escape.[104]

3 | ANTIMICROBIAL PEPTIDES

AMPs are typically small oligopeptides with less than 50 amino acids.[105] Because of their role in the innate immune response, they are often referred to as host defense peptides (HDPs).[106,107] Depending on their secondary structure, AMPs can be categorized into three different groups: α-helical, β-sheet, and extended peptides. The majority of AMPs belong to the first two groups.[108,109] They typically consist of basic amino acids and hydrophobic residues and form unique secondary structures. In particular, they are at the same time water soluble, positively charged, and hydrophobic. This combination of properties is called amphipathic and allows AMPs to interact in a specific way with cell membranes, leading to their capability of killing bacterial and mammalian cells.

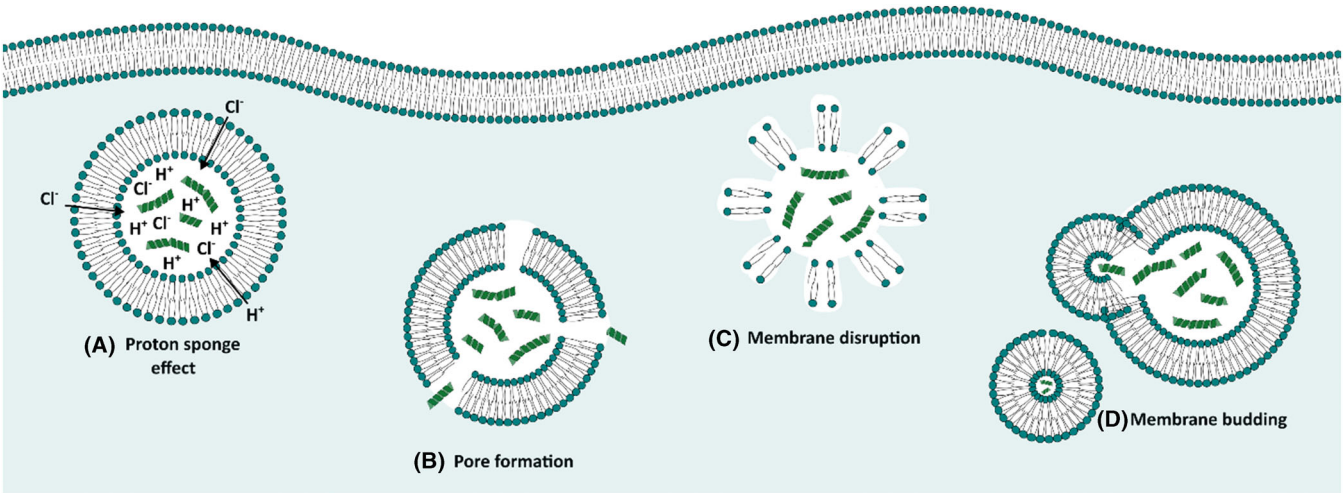


FIGURE 6 Endosomal escape mechanisms—(a) proton sponge effect; (b) pore formation; (c) membrane disruption; (d) membrane budding

Consequently, AMPs are of high interest in pharmaceutical research and bear the potential of replacing classical antibiotics.[110] By investigating their antimicrobial potency, some AMPs like the amidase Pal were found to induce cell death of *Streptococcus pneumonia* within seconds after initial contact with cell membranes,[111] whereas others are not directly affecting bacterial cell viability but are able to synergize with antibiotics and enhance their efficiency.[112] For instance, the combination of the antibiotic penicillin G and various AMPs like pediocin, nisin Z, or colistin lowered the minimum inhibitory concentration (MIC) by several magnitudes. Through this combination, originally penicillin-resistant cells were susceptible to penicillin G again.[113] These effects are ascribed to the specific interactions of the AMPs with the cell membrane that assist permeation of the antibiotic agents and therefore increase their efficiency.

Interactions of AMPs with membranes include disruption,[114] translocation,[115] and permeabilization.[116] The first step in each of these mechanisms is the adsorption of the AMP onto the outer membrane surface. This causes a state of imbalance between the inner and outer lipid layers to which the AMP is bound. This imbalance is thermodynamically unfavored and results in a driving force to distribute the peptides equally between the two monolayer leaflets. AMPs can realize this process following two different pathways. The first possibility is observed mostly for hydrophobic peptides, which can be partly incorporated into the lipid bilayer and form a thermodynamically stable intermediate transmembrane state. In this case, the AMPs translocate across the bilayer without causing a leakage of the cell interior (Figure 7).

The more renowned feature of many AMPs is their ability to disrupt prokaryotic membranes through pore formation in the lipid bilayer. When a critical peptide/lipid ratio is reached, hydrophilic AMPs insert into the membrane, forming a porated lamellar phase (poration) because of lipophobic interactions. These AMPs can undergo three different disruptive mechanisms, namely, the barrel-stave pore, the toroidal pore, and the carpet/detergent mechanism (Figure 4). Independent from the pathway, all these disruptive interactions lead to leakage of the cell interior ultimately causing cell death.[117,118]

Also, peptides that are not disrupting the membrane are classified as AMPs if they induce cell damage by acting on an intracellular target. Such derivatives were reported to impact both prokaryotic and eukaryotic cells.[119–121] For instance, the peptides can be translocated across the membrane and subsequently inhibit intracellular processes, such as DNA replication or interaction with adenosine 5′-triphosphat (ATP), by specific enzymes involved in the regulation of these processes.[122–124]

3.1 | Possibilities to modify AMPs

Because of their versatility, many new AMPs were isolated and investigated, and structural analogs modifying certain properties have been synthesized in recent years.[124] To be able to interact with the membrane, most AMPs are amphipathic, having a cationic and a hydrophobic face. Through electrostatic interaction with the negatively charged bacterial cell membrane, initial contact of the AMP is ensured, which can lead to the insertion into the membrane interior. Because of the hydrophobic part, the AMP can embed further into the lipophilic part of the membrane and disrupt or penetrate it (Figure 7). The same mechanism can be found for interacting with the plasma membrane and for affecting the cell walls of organelles, including the nucleus.[125] Consequently, introducing synthetic modifications affecting the net cationic charge of AMPs allows to change their antimicrobial and hemolytic activities to, for instance, selectively kill microbes. The antimicrobial activity is a measure for the potency of killing microbes, whereas the hemolytic activity is a measure for killing red blood cells. Therefore, a high antimicrobial and simultaneously low hemolytic activity are crucial factors in AMPs, which are considered to be used as antibiotic replacement. However, the two properties are often related to similar structural motifs, and thus, chemical design of suitable peptides is still an unresolved challenge. For example, it was found that raising the net charge of the AMP V13K (acetyl-KWKSFLKTFKSAKKTVLHTALKAISS-amide; Figure 8), from +8 to +9 by exchanging a serine with a lysine residue resulted in a 32-fold higher hemolytic activity. In contrast, lowering the net charge below +4 by exchanging lysine with serine residues results in totally preventing the hemolysis, but the antimicrobial activity against *P. aeruginosa* was negligible.[11]

Another way to synthetically modify AMPs to improve their activity is to change the helicity of α-helices. In this manner, researchers

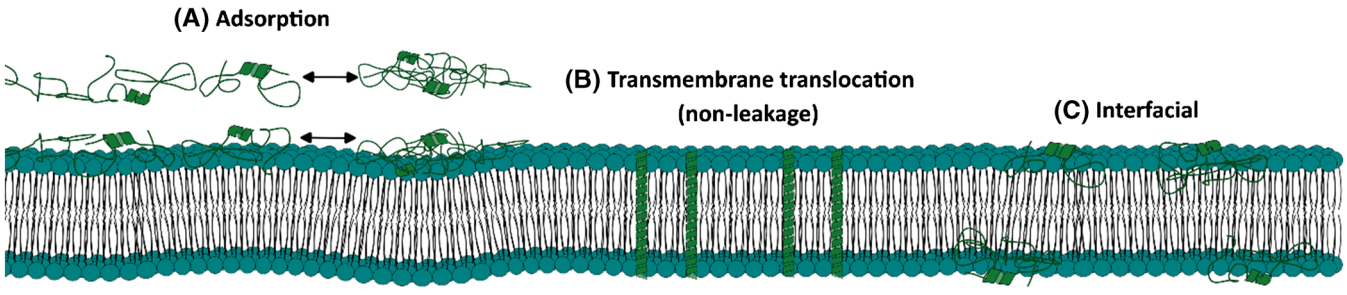


FIGURE 7 Peptides on the outside of the membrane can undergo aggregation prior to adsorption onto the outer membrane layer (a). If the AMPs are hydrophobic enough, translocation will proceed without leakage and cell death (b). This way peptides distribute on both faces of the plasma membrane (c).[117]

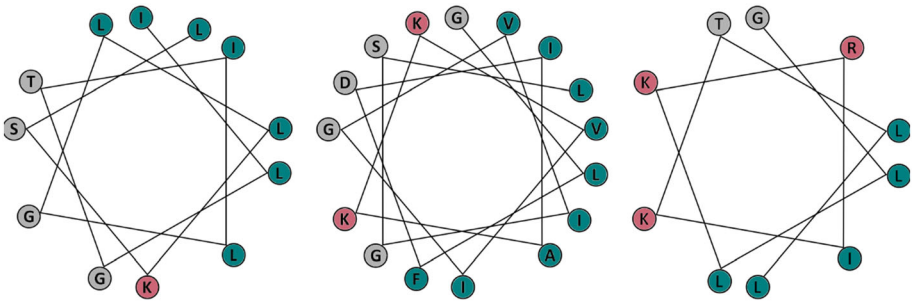


FIGURE 8 Helical diagrams and detailed overview of main representatives of AMPs classes. D-amino acids are abbreviated with AA_D.

Name	Sequence	Modification
V13K	KWKSFLKTFKSAKKTVLHTALKAISS	Charge Alteration
Pardaxin	GFFALIPKIISSPLFKTLLSAVGSALSSSGGQE	Helicity Alteration
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	Helicity Alteration
Amphipathic-1L	$LK_DLLK_DKL_DL_DKKLL_DKLL$	Stereoisomers
KSLK	$K_DK_DVVFKVKFK_DK_D$	Stereoisomers
Polybia-CP	$ILGTILGLLK_DSL$	Stereoisomers
Aurein 2.6	GLFDIAKKVIGVIGSL	Amidation
Aurein 3.1	GLFDIVKKIAGHIAGSI	Amidation
PMAP-23	RIIDLLWRVRRPQKPKFVTVWVR	Amidation
Amurin-9KY	FLPFFAACAITRKC	Amidation
Anoplin	GLLKRIKTLL	Lipidation
L6K6	LKKLLKKLLKKL	Lipidation
F2I-LL	$LRLRFF_DPGIIRLRL$	Self-assembly/Hydrogel
WMR2PA	WGIRRILKYGKRSAAAAAAK-(C19)	Self-assembly/Hydrogel
CaLL	KWKLFKKIFKRIVQRIKDFLR	PEGylation
Ruthenocene-derived	Rc-WRWRWR	Organometallic AMPs

modified the motif of the natural polypeptide toxin pardaxin (GFFALIPKIISSPLFKTLLSAVGSALSSSGGQE; Figure 8). Compared with the similar AMP melittin, pardaxin shows a higher antimicrobial but lower hemolytic activity. The main difference between the two AMPs lies in the charge of the C-terminal region, where melittin is positively charged and pardaxin negatively. Adding a positive charge in the C-terminal region of pardaxin by extending it with $[NH(CH_2)_2NH_2]$ not only increased the helicity by stabilizing the helix dipole moment, but also showed improved antimicrobial and higher hemolytic activities, comparable with the properties of melittin. The findings suggested that the higher activity arose from the 40% increased helicity compared with natural pardaxin. Indeed, observation showed a general trend when they synthesized various pardaxin-derived analogous and aminated them at the C-terminus, which improved both the helicity by 25%–80% and the potency by 2–10 fold.[12]

To influence the helicity of the peptides, L-amino acids were exchanged with their enantiomers in the sequence of the synthetic AMP amphipathic-1L (LKLLKKLLKKLLKLL; Figure 8). Specifically, exchange of five amino acids in different positions deactivated the hemolytic effect completely. Importantly, the antimicrobial activity of amphipathic-1L was preserved. With the exchange of five amino acids (LKLLKKLLKKLLKLL; Figure 8), the α-helicity changed from 79% to 43%.[126] Focusing on a similar strategy, another group showed that replacing two amino acids at both termini of the sequence of KSLK (KKVVFKVKFKK; Figure 8) with D-amino acids retained the antimicrobial potency while lowering the α-helicity by 43%. Furthermore, replacing the central amino acid resulted in disrupting the α-helix and switching off the antimicrobial effect. This allows to conclude that the α-helix was a crucial part of the antimicrobial activity of AMPs, but once formed, the grade of helicity does not have much impact.[127] Others could prove that changing a single L-lysine in polybia-CP (ILGTILGLLKSL; Figure 8) to the D-amino acid lowered the α-helicity content by half (to 12.5%), retained the antimicrobial potency, and reduced the hemolytic activity significantly.[13]

In contrast to lowering α-helicity, it was found that exchanging the C-terminal acid group with an amide, in this context referred to as amidation, in fact increases the α-helicity of the AMP aurein 2.6 and aurein 3.1 by 10%–40%.[128] This represents an alternative modification in the peptide sequence that typically has a major impact on the AMPs. This was further investigated using the AMP PMAP-23 as an example. The researchers found that the derivate had a different orientation inside the membrane in comparison with the original PMAP-23. Additionally, this alteration improved the cellular uptake of the compound by almost 10-fold, resulting in a faster interaction with the cell membrane and a

deeper incorporation into the membrane.[129] Similar conclusions were found by others while investigating the C-terminal amidation of the AMP amurin-9KY (FLPFFAACAITRKC; Figure 8). As the natural amurin-9KY could not show any lethality against bacterial cells at concentrations up to 2 mg/ml, the synthetically modified amurin-9KY1 with amidated C-terminus introduced the ability to kill various cells at the same concentration. In addition, the hemolytic activity was reduced by 26% compared with the natural amurin-9KY.[130]

Also, larger structural modifications can be used to impact the antimicrobial activity of AMPs. In this context, lipidation refers to the synthetic modification of AMPs by attaching fatty acids mostly to the N-terminus or lysine residues. This modification is used to stabilize the secondary structure of the peptides and promotes the association with cell membranes. Furthermore, it leads to increased folding of short peptides in general.[131] Lipidation can enhance the antimicrobial activity and the tolerance of AMPs against proteases by increasing hydrophobicity. If the attached fatty acid is too long, however, the bioactivity of the peptide decreases because of the reduction of the α-helical content. There is a certain threshold for increasing α-helicity and antimicrobial activity by raising the hydrophobicity of AMPs. Raising the hydrophobicity above the threshold resulted in decreasing bioactivity, as the researchers showed with the AMP anoplin (GLLKRIKTLL; Figure 8). They attached fatty acids (C_4–C_{12}) N-terminally to anoplin and found increased microbial activity and resistance against proteases. However, from C_{10} upward, the effects started to diminish.[132–134] Furthermore, with longer acyl chains, the AMP becomes prone to self-assembly, which decreases the peptide–membrane interaction. Also, the effect of lipidation on the short synthetic AMP L_6K_6 (LKKLLKKLLKKL; Figure 8) was investigated. The parental AMP had insufficient hydrophobicity and thus low bactericidal properties because of low membrane binding affinity. The attachment of fatty acids (C_{10}–C_{16}) increased the hydrophobicity to a point, where AMPs with 10–12 additional carbon atoms showed good antimicrobial activity (four to eight fold higher). With 14–16 added carbon atoms, however, the bactericidal effect dropped to the level of the parental AMP. Circular dichroism spectroscopy revealed that the reason for oligomerization of AMPs with longer fatty acid chains is due to micellization of the hydrophobic alkyl chains.[135]

On the other hand, self-assembly can also be exploited to impact antimicrobial potency positively. For instance, researchers synthesized the AMP F2I-LL (Ac-LRLRFF$_D$PGIIRLRL; Figure 8), which can self-assemble into nanofibers at higher concentrations (16 μM) to eventually form a biocompatible hydrogel (Figure 9). The hydrogel showed an improved antimicrobial activity of four to 10 times compared with the same AMP in its monomeric form and exhibits a membrane-disruptive mechanism. In addition, F2I-LL showed no hemolytic activity up to a concentration of 128 μM.[136] Also, in the peptide WMR2PA (NH_2-WGIRRILKYGKRSAAAAAAK[C_{19}]-$CONH_2$), self-assembly has a

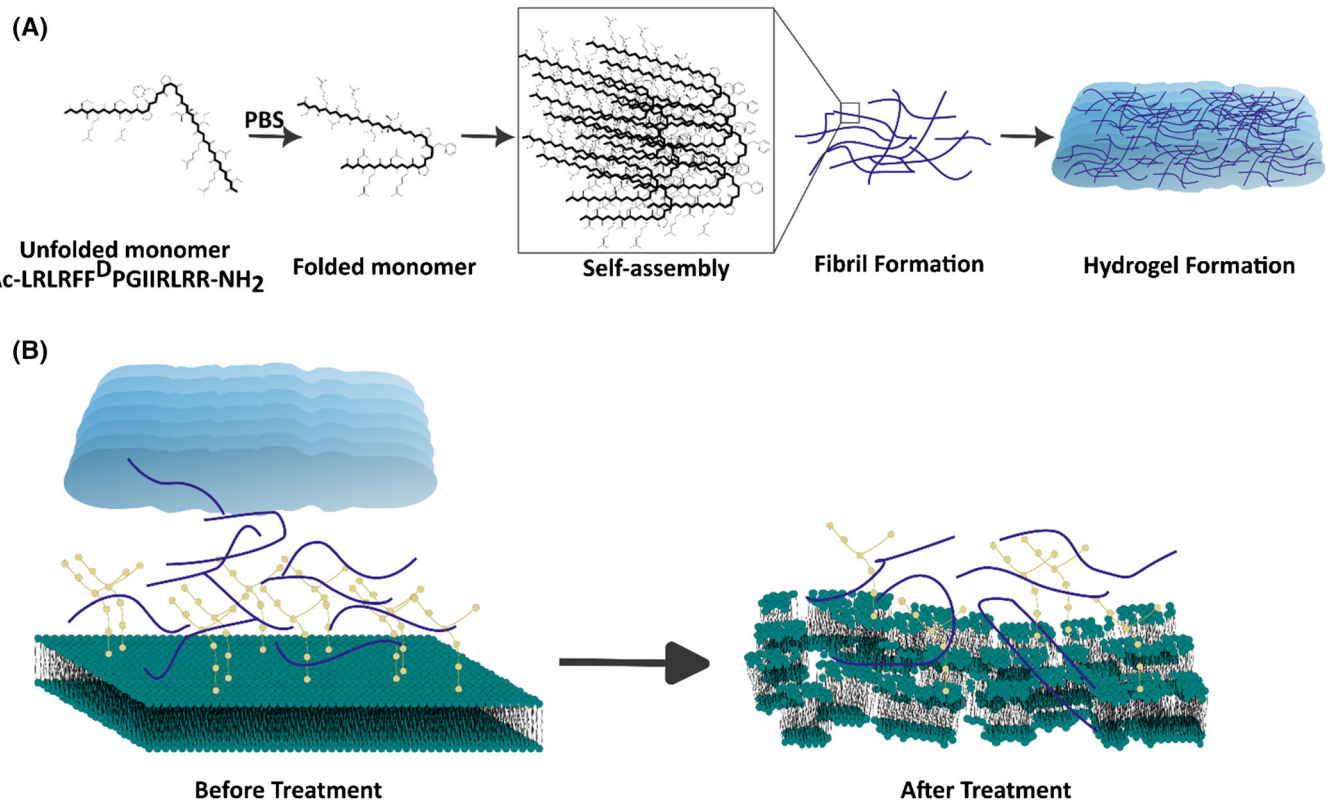


FIGURE 9 Pathway of the folding, self-assembly, and hydrogel formation of the AMP F2I-LL. The initial folding is induced by phosphate-buffered saline (PBS). Self-assembly occurs above a concentration of 16 μM because of chemical complementarity and structural compatibility of the peptide chain. Treating bacterial cells with the hydrogel leads to cell death through a membrane disruptive mechanism by forming pores and resulting in cell leakage.[136]

beneficial effect on its antimicrobial potency. WMR2PA is a synthetic AMP that is derived from the natural peptide myxinidin and contains six additional alanines, a lysine, and a lipidic tail ($C_{19}H_{38}O_2$). The latter enables the self-assembly of WMR2PA into nanofibers in aqueous environment. In these, the lipidic tails form the core, whereas the biologically active peptide part of the AMP faces outward. The authors investigated the prevention and eradication of biofilm formation of *P. aeruginosa* and *C. albicans*. They found that the self-assembled WMR2PA was equally or less effective against biofilms of *P. aeruginosa* compared with the monomeric analog WMR (NH_2-WGIRRILKYGKRS-$CONH_2$). However, in biofilms of *C. albicans*, they found higher potency of the self-assembled form. Further experiments showed that the peptidic part of the construct could be shortened or modified to enhance the activity against biofilms of both *P. aeruginosa* and *C. albicans*, whereas the lipidic tail and the thus induced self-assembly were crucial for the biological activity and could not be omitted. The researchers concluded that this could serve as a designing tool for AMPs.[137]

A different modification is the attachment of polyethylene glycol (PEG) to the peptide strand. It was shown that conjugating a PEG chain to the N-terminus of the AMP CaLL (KWKLFKKIFKRIVQRIKDFLR) results in an increased biocompatibility while retaining membrane binding sites, but 50% decreased antimicrobial activity.[138] Similar results were found by others when PEGylating the C-terminus of various AMPs (aurein 2.1, 2.6, and 3.1). In this example, both the bactericidal activity and the hemolytic activity were reduced from 10% to 3% in the mentioned examples.[139]

AMPs can also be conjugated to organometallic agents (OMs). These OM-AMP variations show increased antimicrobial activity. One OM-AMP is the ruthenocene (Rc)-derived Rc-WRWRWR (Figure 8). The peptide interacted differently with the bacterial membrane and caused biophysical changes, such as lowering the binding affinity of membrane-bound proteins like the vital enzyme MurG and cytochrome *c*. Especially, the removal of MurG affected the antimicrobial activity of the AMP as it prevents the formation of cell walls by interfering in the transformations of lipid I to lipid II, as shown in Figure 10, and thus made the bacteria more vulnerable to external molecules penetrating the membrane.[140]

AMPs cannot only be structurally modified to increase their potency toward bacterial cells but they can also be combined with other molecules or targeting moieties to adjust for the type of target cell. For example, conjugating AMPs to a histidine (HIS)-rich vector, that is, GHHPH, resulted in a cancer cell-sensitive AMP. The AMP used here was the short repetition unit of $(RW)_3$ because it showed good cytotoxic effects after intracellular delivery. The vector GHHPH was derived from a plasma protein (histidine-rich glycoprotein HRGP), which showed two ways of acquiring cationic charges: at pH values below normal physiological condition the histidine would protonate and the vector could chelate zinc ions. These capabilities are important for targeting cancer cells because the microenvironment of a solid tumor is acidic and shows increased concentrations of zinc ions. In particular, the positive charge from this HIS vector can initiate electrostatic interactions with glycosaminoglycans that are often overexpressed in cancer cell membranes. For this project, the targeting vector and the cytotoxic peptide were linked by a cysteamine-cysteine disulfide linker system.[141]

4 | MEMBRANE COMPOSITION/ MORPHOLOGY CHANGES INDUCED BY PEPTIDE INTERACTIONS

Peptides, such as CPPs and AMPs (vide supra), interact with lipid bilayers in order to disrupt or penetrate them. In the previous sections,

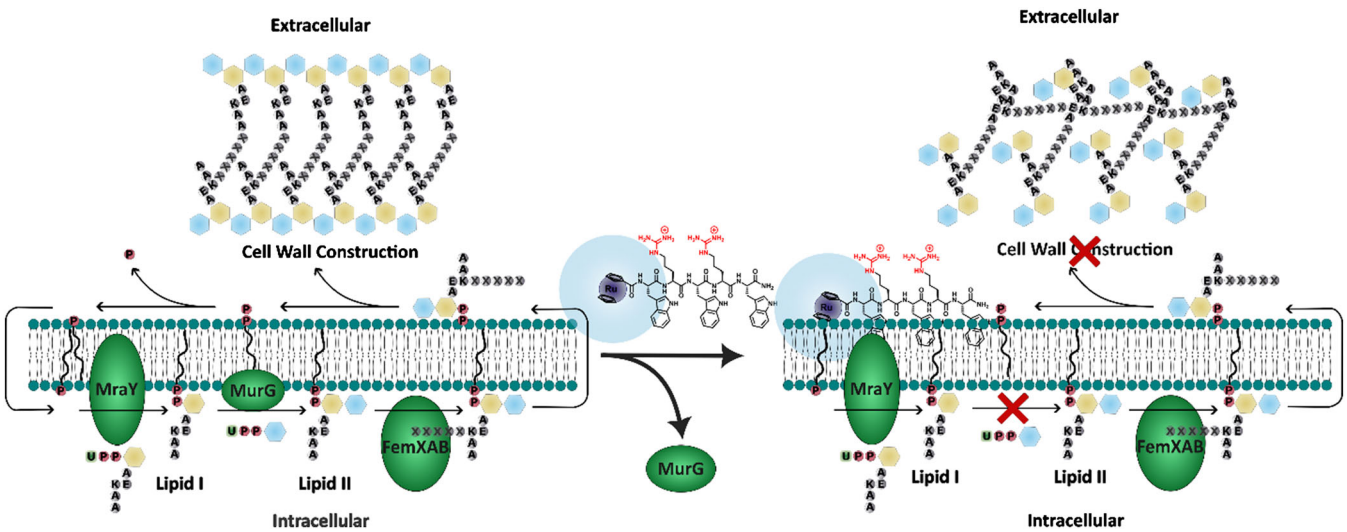


FIGURE 10 Shown on the left is the normal pathway of constructing the cell wall. The lipids are transformed and transferred from MraY to the next enzyme MurG and finally to FemXAB, till the cell wall is constructed in the end. With the attachment to the membrane and the interference of the OM-AMP, the MurG enzyme is removed from the membrane and the transformation of the lipid I → II is inhibited. This results in the accumulation of only lipid I, which ultimately prevents new cell wall formation.[140]

we discussed the different pathways and mechanisms of these peptides to interact with cell membranes. In contrast, in this chapter, we will focus on the consequences of peptide-lipid interaction for the composition and morphology of the cell membrane.

One parameter that is affected as a result of the interaction between peptides and a cell membrane is the fluidity of the membrane. This phenomenon was discovered while investigating the different ways of membrane perturbation of the three AMPs TC19 (LRCMCIKWWSGKHPK), TC84 (LRAMCIKWWSGKHPK), and BP2 (GKWKLFKKAFKKFLKILAC). They measured changes in the membrane fluidity through fluorescence spectroscopy and the usage of laurdan (6-dodecanoyl-*N*,*N*-dimethyl-2-naphthylamine), a fluorescent probe sensitive to membrane phase transitions. The otherwise rigid membrane showed domains, in which the fluidity was significantly increased.[142] These domains mainly differ in their lipid phase (liquid-ordered L_o/liquid-disordered L_d) and therefore in rigidity.[143] The higher affinity for anionic phospholipids to the cationic AMPs likely resulted in an accumulation of the AMPs in areas associated with these lipids. Subsequently, the accumulated AMPs align themselves parallel to each another and insert into the membrane to cause "free volume" in the bilayer. The lipid layer then compensates for this new volume by membrane thinning and this leads to increased fluidity. The researchers proposed that the so-formed domains of higher fluidity could act similarly to pores resulting in leakage of cell interior material and were the origin of the antimicrobial property of these AMPs (Figure 11).[142]

Lipid domains can be observed in diverse biological processes. For example, domain formation in the cell membrane is involved in the immune response or in communication of the cell by creating signals.[144–146] The possibility of artificially forming these domains was investigated by introducing the α-helical (AH) domain of the hepatitis C virus protein NS5A as a synthetic virocidal peptide into a lipid bilayer. The study showed that the AH peptide exhibited different binding affinities to the membrane, depending on the membrane composition. In the presence of phase-separating membrane components like sphingomyelin or cholesterol, the binding of the AH peptides would induce a pronounced redistribution of the lipid. This rearrangement then led to the formation of distinct lipid domains. Control experiments showed that in the absence of the phase-separating agents, the AH peptides would not have any effect at comparable concentrations. At higher concentrations, a softening of the membrane was observed, which did however not induce any domain formation.[19]

On the other hand, on a membrane, which already formed phase-separated domains, amphipathic peptides are likely to accumulate at the outer edges of the L_o phase.[147] The hydrophobic core of the L_o domains is thicker compared with that in the surrounding L_d phase. This leads to a hydrophobic mismatch similar to the membrane thickening effect (vide infra). This difference is compensated for by elastic deformation, which in turn induces an increased curvature in the membrane. Because amphipathic peptides mostly induce local curvature, they prefer areas in a lipid bilayer with the same property, such as the boundaries between the two phases L_o and L_d. The incorporation of amphipathic peptides at the phase separations results in a repulsive interaction between the peptides of different domains. This impends the fusion of smaller domains, resulting in a disturbance of normal cell functions.[146]

Similar results were found when investigating five different AMPs (Figure 8). Three of them (Mag2, GS, and BP100) showed membrane-thinning effects after interacting with the lipid bilayer. The authors explained that the thinning arises from only partial incorporation of the peptides into the membrane and thus creating more area on the outer leaflet of the bilayer. This causes a reduction of the hydrophobic layer thickness. Interestingly, the same AMPs showed no or only poor ability to incorporate and tilt inside the membrane.[20,148] On the other hand, PGLa, an AMP with a similar structure as Mag2, tilts when entering the membrane and does not affect the membrane thickness. PGLa also incorporates completely and thus displaces fewer lipids. The fifth derivative of the series, TisB, showed membrane thickening via dimer formation inside the membrane. This proceeds without tilting as the hydrophobic core faces the inner membrane region. The hydrophobic moiety of TisB (43.5 Å) is longer than the hydrophobic interior of the lipid bilayer used in this study (DMPC, 22.8 Å). As a result, the membrane thickens to reduce this length mismatch.[20]

Besides lipid bilayer thinning and thickening, also the curvature of the membrane is an important aspect regarding its morphology. In biological processes like endocytosis and vesiculation, a change of the membrane's curvature has been observed and plays a crucial role in

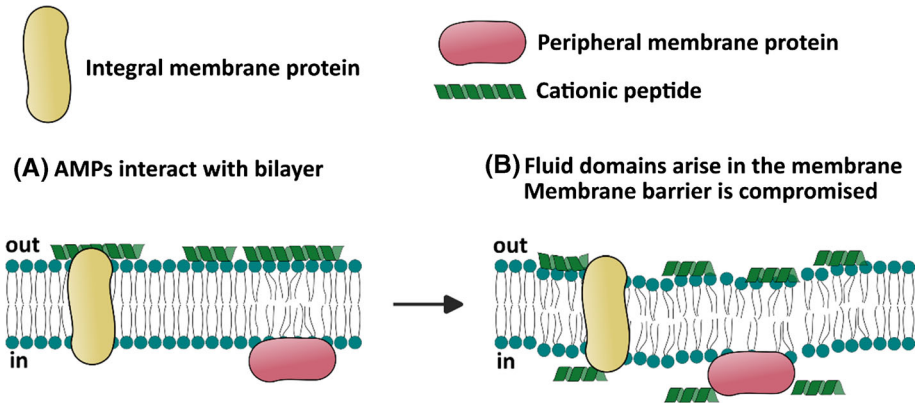


FIGURE 11 (a) Accumulation of the AMPs on areas associated with anionic phospholipids. The peptides either incorporate into or align parallel onto the membrane. (b) The increased peptide concentration creates "free volume" that is compensated for with membrane thinning. This leads to locally increased membrane fluidity, which in return causes pore-like behavior including leakage of cell interior, and delocalization of essential membrane-bound proteins.[142]

them.[149] Researchers synthesized the amphipathic helical peptide EpN18 (XSTSSLRRQXKNIVHNYS–amide, X = norleucine) that derived from the N-terminus of the protein epsin-1. They found that the incorporation of EpN18 in HeLa cells led to packing defects in the membrane (Figure 12). The peptide was incorporated only superficially in the outer lipid layer, which resulted in a bending of the inner leaflet. Interestingly, an EpN18 trimer connected to an Ac–KGKGKG backbone had a dramatically increased effect as the local concentration of the peptide increased. Thus, the minimum concentration of EpN18 required to observe curvature and packing defects decreased from 40 to 0.5 μM. This increased curvature of the membrane was accompanied by an improved uptake of CPPs, like the synthetic octaarginine (R8). In the presence of 0.5 μM of the EpN18 trimer, the minimum required concentration of R8 to penetrate the membrane was reduced by 50% to 10 μM.[150]

One of the most important factors in the context of membrane composition or morphology is cholesterol. It plays a crucial role in membrane curvature and lipid domain formation and is an essential component of the membrane in mammalian cells.[151,152] Another important aspect of cholesterol in the outer membrane is its influence on membrane fusion and fission (vide infra).[153] It was proposed that because of inducing a negative curvature in membranes, cholesterol lowers the energy for forming lipid stalks. These stalks are supposedly intermediates in membrane fusion.[154,155] Furthermore, increasing the cholesterol concentration results in a more efficient SNARE-mediated membrane fusion.[156–158] This shows the existing link between membrane composition and the efficiency of membrane fusion peptides.

5 | FUSION PEPTIDES

Another way peptides can induce a change in membranes is by initiating the fusion of two separate membranes into one.[159] Membrane fusion is an important process involved in, for instance, the communication between different organelles and cells.[160] The most studied of these processes is exocytosis, which is based on the fusion of a vesicle, that was formed inside the cell, with the outer cell membrane. The merging of the two membranes leads to the release of the contents of the vesicle, such as hormones or neurotransmitters, outside the cell.[161] Because of the high importance of membrane fusion, the process is tightly regulated and relies, inter alia, on a class of mediating peptides that is commonly referred to as fusion peptides. This type of peptide is not only present in eukaryotic cells but is also used by enclosed viruses to gain entrance into the targeted host cell.[159] Even though fusion processed in eukaryotes and prokaryotes does have mechanistic resemblances (Figure 13), the viral fusion peptides do

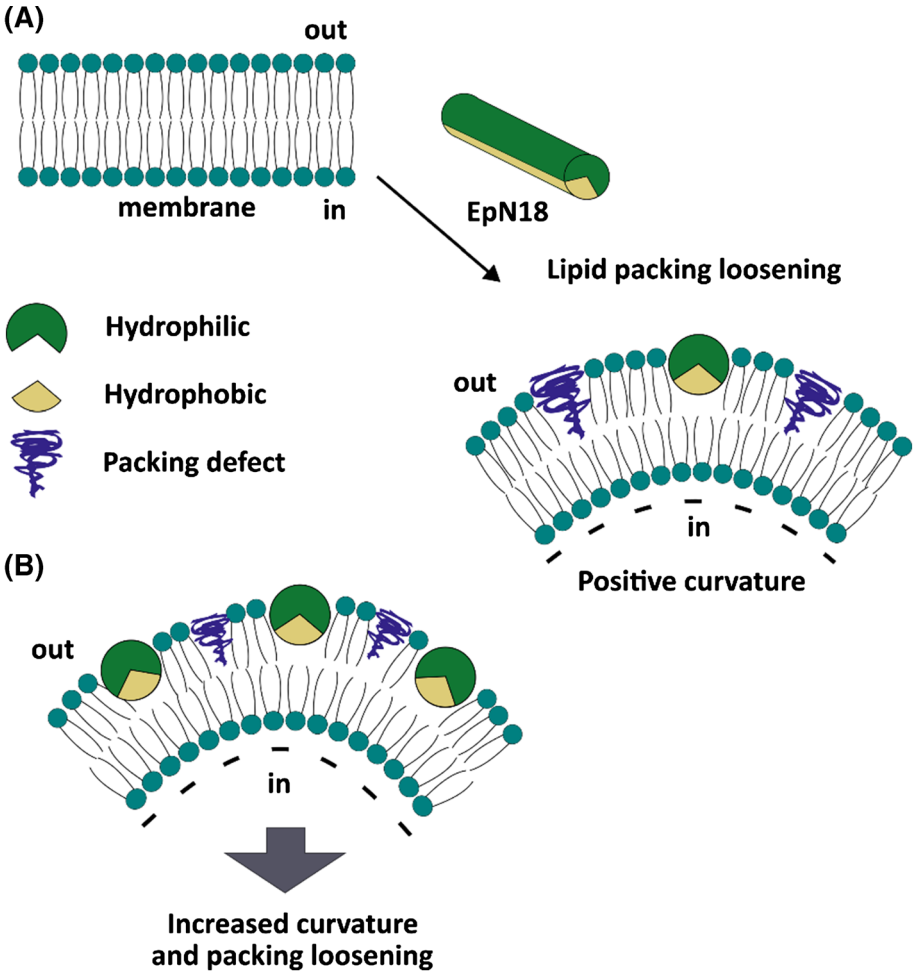


FIGURE 12 (a) EpN18 is an amphipathic peptide derived from the N-terminus of the protein epsin-1. Incorporation of EpN18 induces loosening of lipid packing and a positive curvature of the membrane. (b) Trimerization of the peptide leads to increased local concentration and therefore enhances the effects described in (a).[150]

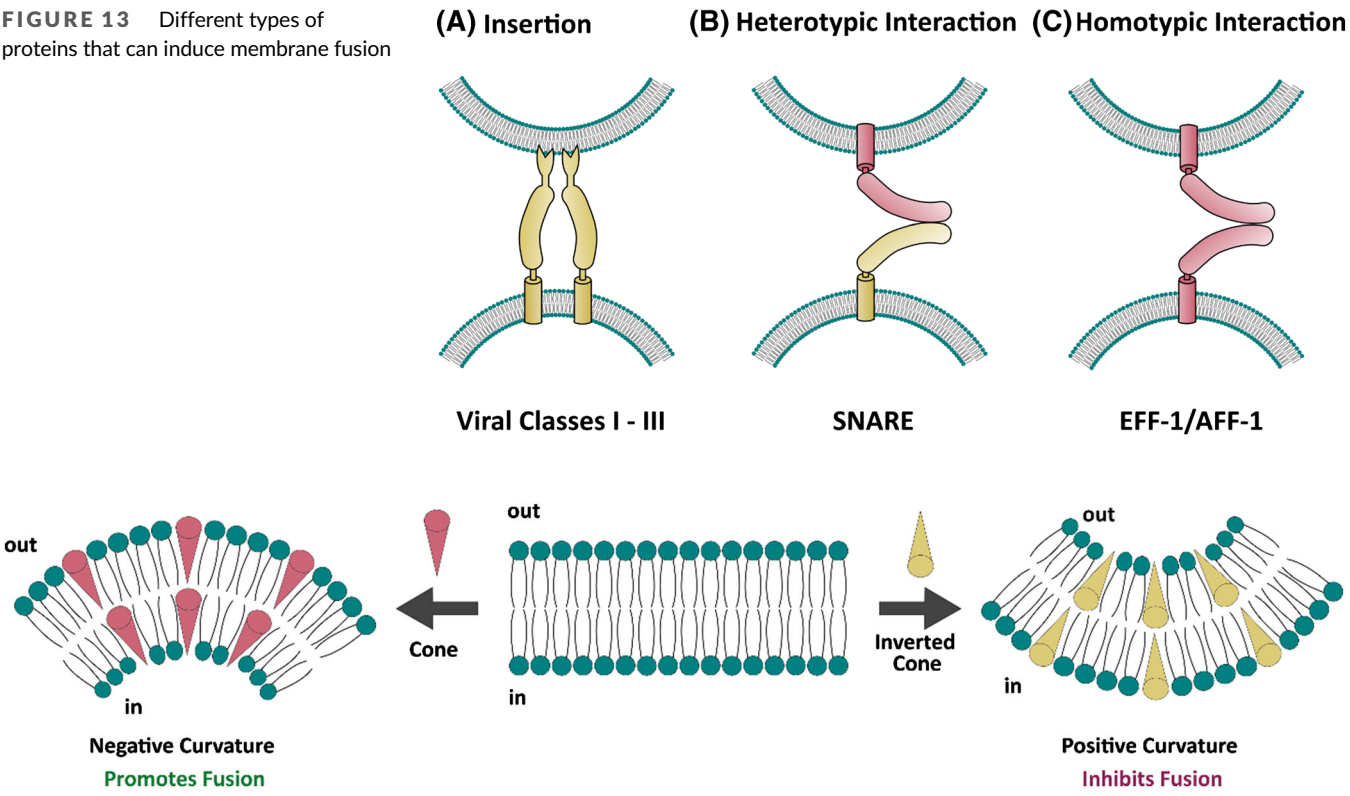


FIGURE 13 Different types of proteins that can induce membrane fusion

FIGURE 14 The effect of different shape phospholipids on membrane curvature[164]

show a greater variety in the modes of action (vide infra) and can serve as a great inspiration for artificial systems.[162] Despite the similarities in the fusion processes of eukaryotes and viruses, most synthetic models have been used to study eukaryotic fusion peptides and the mechanisms.

During membrane fusion, the content of the previous two compartments is shared. In the same manner, the two different lipid bilayers merge, and thus, the phospholipid content of the emerging vesicle and with that its morphology can be altered by the peptide-mediated fusion process. However, also the membrane composition itself can have an effect on the efficiency of the fusion peptides. For instance, it has been shown that incorporation of certain phospholipids that affect the curvature of the lipid bilayer affects the efficiency of fusion.[163]

Both fusion efficiency and curvature are closely linked since lipid bilayers with a higher curvature are energetically more strained. This leads to a lower energy barrier for membrane fusion as the curvature, and thus, the strain of the membrane is reduced after fusion. Therefore, incorporating phospholipids that are known to increase the curvature of lipid bilayers improves the efficiency of fusion peptides (Figure 14).[164,165] Also, cholesterol, which is present in significant amounts in eukaryotic cells, has been shown to promote fusion.[166] Selective removal or enzymatic modification of cholesterol was found to reduce Ca^{2+}-sensitive synaptic vesicle fusion efficiency. Furthermore, addition of external cholesterol to these depleted membranes showed recovery of fusion as well as Ca^{2+} sensitivity. Although the addition of membrane components leading to a similar membrane curvature as cholesterol also recovered fusion efficiency, the same did not happen to Ca^{2+} sensitivity. Therefore, it can be deduced that cholesterol can promote membrane fusion both by creating negative membrane curvature and interacting with protein factors that regulate Ca^{2+}-triggered membrane fusion.[154] Further studies have shown that higher concentrations of cholesterol lead to direct opening of the fusion pore, whereas in lower cholesterol concentration, a prolonged hemifusion intermediate is formed.[157,158]

5.1 | Viral membrane fusion

Enveloped viruses utilize membrane fusion to enter and infect eukaryotic cells. These processes are mediated by viral fusion proteins that are glycosylated and anchored in the viral envelope. Contrary to eukaryotic fusion proteins, where the fusion process is initiated by the interactions between the fusion proteins themselves, viral fusion is triggered by an external environmental stimulus, such as acidic pH or binding to a coreceptor.[162]

This trigger induces a conformational change in the viral fusion protein that allows the native "fusion competent" protein located on the viral membrane surface to be embedded into the host membrane. This results in the "pre-hairpin" conformation containing a hydrophobic segment referred to as the fusion peptide that inserts itself into the host membrane. This insertion destabilizes the lipid bilayer of the host cell, leading to a reduction in the energy required to induce fusion. Afterwards, further conformational changes lead to folding of

the fusion protein, which brings the two membranes in closer proximity and initiates hemifusion eventually leading to full fusion by creating the fusion pore (see Figure 15).[14,167] Although this is the general mechanism of action, viral fusion proteins are classically divided into three distinct classes based on their structural features. However, studies have shown that there are several viral fusion proteins that do not correspond to these three classes.[167]

Class I consists of proteins with three bundled α-helices in the core of the pre-fusion complex, which refolds into a 6-helix bundle after the fusion. This refolding is also the driving force for the membrane fusion because of the creation of a stable rod-like structure of the fusion proteins with a central trimeric coiled coil. Furthermore, the fusion peptides of this class are located at the N-terminus of the viral glycoprotein and are proteolytically released. Coronaviruses, retroviruses, orthomyxoviruses, filoviruses, and paramyxoviruses are all known to contain Class I fusion proteins; however, the most studied examples of this class are influenza virus and human immunodeficiency virus type 1 (HIV-1) fusion proteins.[14,167–169]

Class II differs from Class I viral fusion proteins in their three-dimensional structure. Members of this class of proteins have a three-domain structure that is composed predominantly of β-sheets and the fusion peptide forms a loop located in the central domain, which inserts itself in the host membrane during fusion.[167] The composition of this loop is important for fusion efficiency because substitution with negatively charged amino acids is known to inhibit membrane fusion.[170] Class II fusion proteins also are not proteolytically cleaved, and instead of forming coiled coils, they form homo or hetero-dimers with viral glycoproteins.[171] Furthermore, Class I proteins are perpendicular to the membrane, whereas Class II ones adopt an orientation parallel to the membrane.[16,167]

Lastly, Class III fusion proteins are found in herpesviruses, baculoviruses, and rhabdoviruses and contain characteristics of both previously described classes. They exhibit helical domains similar to Class I and show a central domain with a fusion loop similar to Class II. Furthermore, they are not proteolytically cleaved and do not contain coiled-coil structure.[172] Because of these significant structural differences, they were introduced as a separate class of fusion proteins.[167]

Several viral fusion peptides are known to adsorb onto the host's membrane, which causes disturbance and destabilization and allows for easier entrance of viruses into eukaryotic cells.[165] For example, content leakage assays showed that the SARS-CoV fusion peptide preferentially binds to and destabilizes negatively charged membranes.[14,173]

5.2 | Eukaryotic membrane fusion

A large range of eukaryotic fusion peptides is known up to date and involved in different types of membrane fusion processes in eukaryotic cells. Within this family of peptides, SNAREs are the most commonly studied ones. More than 30 different SNARE peptides can be found throughout eukaryotic cells.[174] This type of fusion peptide acts via two complementary peptides located each on different membranes (Figure 16a). They can then recognize each other and interact forming the SNARE motif (Figure 16b), which brings the membranes in closer proximity to one another. This forced proximity is one of the driving forces to eventually induce membrane fusion (Figure 16c).[16,175]

Even though the general mechanism of SNAREs is well described, not all aspects are fully clarified yet.[176] Thus, several synthetic peptide models based on the native SNARE proteins were developed and used as probes to study SNARE-mediated membrane fusion.[177,178] Specifically, the synthetic models have proven to be useful to understand the role of specific regions of these proteins and to mimic the actions of native proteins using minimalistic systems.

5.3 | SNARE proteins

SNARE peptides consist of three distinct regions, that is, the transmembrane domain that anchors the peptides in the membrane, the SNARE motif that interacts with other complementary SNARE peptides, and the linker region in between (see Figure 17).[18] All three sections of the peptide play a role in the fusion process and structural modifications impact the fusion efficiency. Studies have shown that the attractive interactions between SNARE motifs on the two complementary peptides are the main driving forces for membrane fusion. These interactions are proposed to happen by a zippering process,[180] where the complementary motif aggregation initiates at the N-terminus and continues toward the C-terminus, thus bringing the lipid bilayers closer together. Several synthetic models have been created that mimic the action of SNARE-mediated membrane fusion. Generally, these artificial analogs take inspiration from the natural peptide

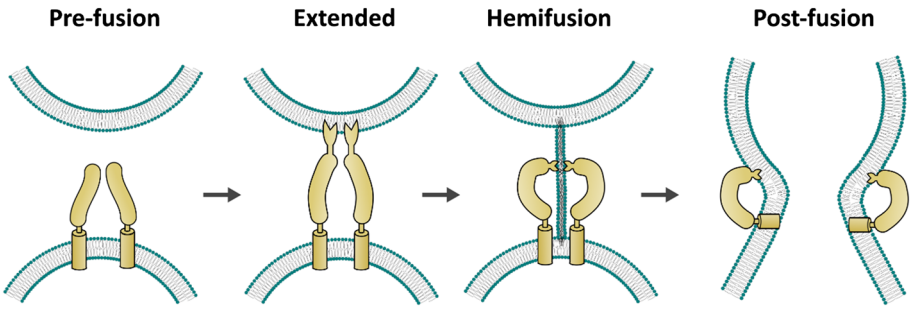


FIGURE 15 Viral insertion-based peptide-induced membrane fusion mechanism[16]

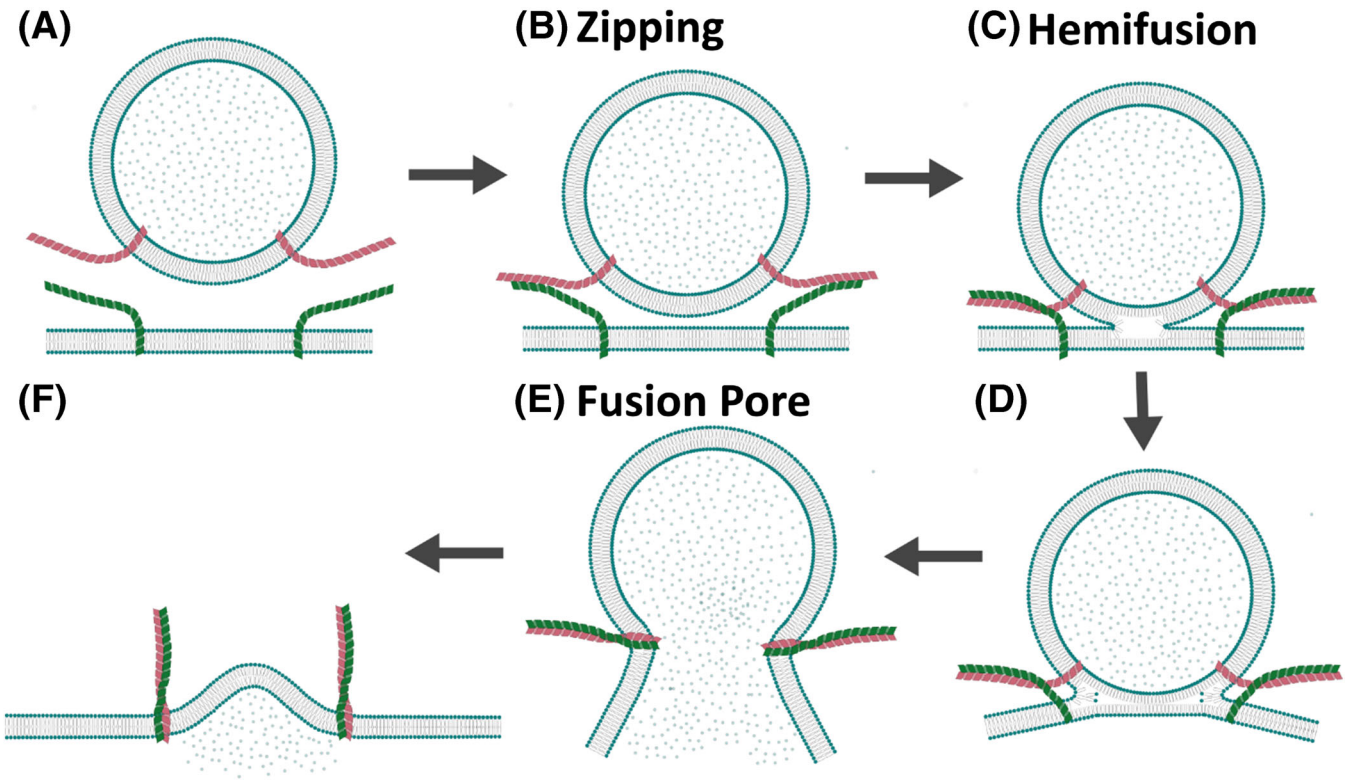
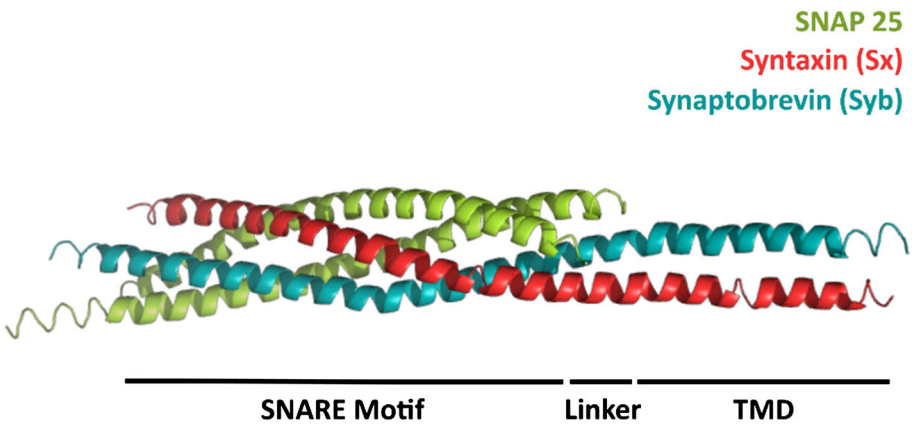


FIGURE 16 SNARE-induced membrane fusion mechanism[175]

FIGURE 17 Natural SNARE protein structure[179]

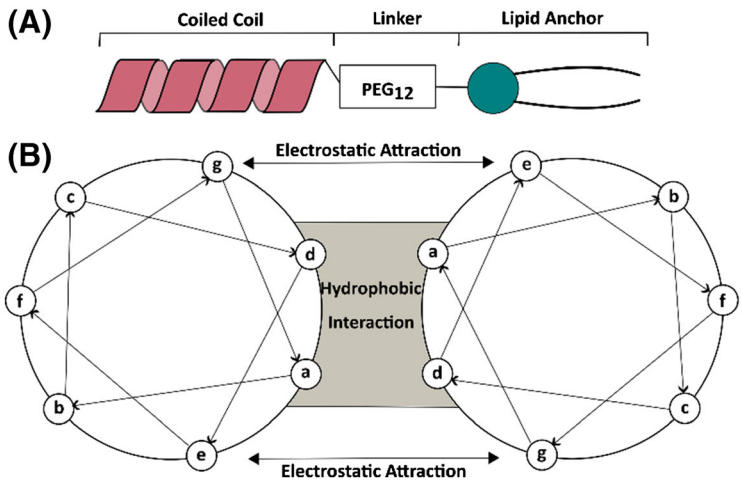


exhibiting a structural motif that anchors in the construct into membrane and a part that mimics the SNARE motif and is capable to interact with a second synthetic SNARE model peptide. In this part of the review, we have compiled a brief overview of the different SNARE-derived synthetic peptides that can interact with membranes and induce fusion. The most used mimics for the SNARE recognition motif are coiled coils.

5.3.1 | Coiled-coil models

Coiled coils are helix-forming peptides consisting of a heptad repeat, which is labeled *abcdef* (Figure 18b). The amino acids at positions *a* and *d* are hydrophobic and have a distance of approximately one helix turn allowing coiled coils to interact through attractive hydrophobic forces.[181] The other positions of the heptad can be varied to introduce additional residues that generate more attractive interactions between coiled coils.[182]

The most common SNARE peptide model utilizes the interactions between coiled coils and is especially attractive because the native SNARE peptide recognition motifs also consist of heptad repeats with the propensity to form coiled coils. The shortest known peptides that form coiled coils are the E3 and K3 (Figure 18c).[182] They are short, relatively to naturally occurring coiled coils, glutamic acid (E)-rich and lysine (K)-rich coiled-coil peptides, respectively, and consist each of three heptad repeats. Since they have opposite charges, they can



(C)

Name	Coiled Coil Sequence $(gabcdef)_4$			
E3	EIAALEK	EIAALEK	EIAALEK	
K3	KIAALKE	KIAALKE	KIAALKE	
E4	EIAALEK	EIAALEK	EIAALEK	EIAALEK
K4	KIAALKE	KIAALKE	KIAALKE	KIAALKE
P2E	EIQQLEE	EIAQLEQ	KNAALKE	KNQALKY
P2K	KIAQLKQ	KIQALKQ	ENQQLEE	ENAALEY
P3E	KNAALKE	EIQALEE	ENQALEE	KIAQLKY
P3K	ENAALEE	KIAQLKQ	KNAALKE	EIQALEY
P1K	KIAQLKE	KNAALKE	KNQQLKE	KIQALKY

FIGURE 18 (a) General structure of the coiled-coil SNARE model. (b) Helical wheel representation of coiled-coil interactions. (c) Structure of different coiled coils[181]

interact with each other via a mix of electrostatic and hydrophobic interactions.

The K3/E3 motif was first introduced in 2009 to determine the minimal machinery required for membrane fusion.[177] In this construct the transmembrane domain of the peptide was replaced with a dioleoylphosphoethanolamine (DOPE) anchor, whereas the flexible linker was substituted with a short PEG chain (Figure 18a). This early study demonstrated that two liposome populations, each decorated with the complementary model peptides, undergo fusion. The fusion process was studied using a variety of analytical techniques including lipid mixing, liposome content mixing, and FRET assays. Furthermore, CD measurements showed a change in the peptide structure because of the interactions between the two complementary coiled coils upon liposome mixing (Figure 18) allowing to conclude that using coiled coils to mimic SNARE proteins meets all the characteristics of the native SNARE membrane fusion.[177]

Further research focused on how the other components of the model, such as the anchor, affect the efficiency of fusion. Interestingly, by using coiled coils with hydrophobic phospholipid anchors, such as DOPE, and cholesterol, content mixing studies showed that these peptides could readily incorporate themselves into already existing vesicles and induce membrane fusion. Consequently, the same constructs were used to induce fusion in pre-existing liposomes. However, the efficiency of the fusion process was decreased because of only partial incorporation of the peptides in the liposomes.[183]

Also, the position of the anchor at the peptide strand has an effect on the membrane fusion. This was, for instance, demonstrated by using cholesterol to anchor E3 and K3 coils into liposomes. Although both peptides were still well incorporated in the membranes when having the anchor at the C-terminus of the peptide similar to previous model systems, as well as in the middle of the peptide chain, the fusion efficiency was affected in all cases. Specifically, the fusion process was impaired most when the anchor was positioned in the middle of the E3 coiled coil. No such trend was observed for the K3 peptide. This could be explained by the different ways these peptides interact with the membranes when they are not actively promoting fusion. Although the fusion of membranes is mediated by the formation of E3/K3 dimers, E3 is also homodimer-prone. Furthermore, K3 is known to interact with the surface of membranes.[184,185] When having the anchor attached to the central part of its strand, E3 can no longer form stable homodimers with other peptides on the same liposome. This leads to the formation of homodimers with peptides on other liposomes, potentially causing aggregation or even content leakage. In contrast, in K3 derivatives that have a centrally positioned anchor, the remaining peptide chain cannot insert itself fully into the membrane they are attached to; thus, K3 remains available for fusion. Therefore, a central anchor on the K3 peptide actually reduces the unfavorable interaction of the peptide for fusion, providing more free K3 peptide that could interact with E3.[186]

The diverse interactions between K3 and E3 peptides with the lipid membrane (Figure 19) were also studied in silico using all-atom simulations and free energy calculations. It was shown that both peptides can adsorb on the lipid membrane surface. However, K3 is able to penetrate deeper into the bilayer because of electrostatic interactions with lipid phosphates as well as the longer length of the lysine hydrocarbons compared with glutamic acid.[188] These findings were later confirmed by fluorescence, CD, and NMR spectroscopy as well as by molecular dynamics (MD) simulations. Especially, in solid state ^{31}P-NMR, it could be visualized that the insertion of the K3 peptide changes the local curvature of the membrane and reorganizes the membrane composition, both of which are effects beneficial for fusion.[187] This reorganization of the membrane composition was also found previously in MD studies showing that K3 increases the amount of cholesterol in the vicinity of the peptide by 10%. As cholesterol is one of the lipids that increase the membrane curvature (vide supra), fusion efficiency is increased in this manner.[189]

Further, MD simulations investigated the effect that different membrane compositions have on the absorbance of the fusion peptides focusing on neutral phosphatidylchloride (PC)/phosphatidylethanolamine (PE)/cholesterol bilayers and negatively charged phosphatidylglycerol (PG)/egg sphingomyelin (eSM)/cholesterol bilayers. It was shown that charged phospholipids, such as PG, increased

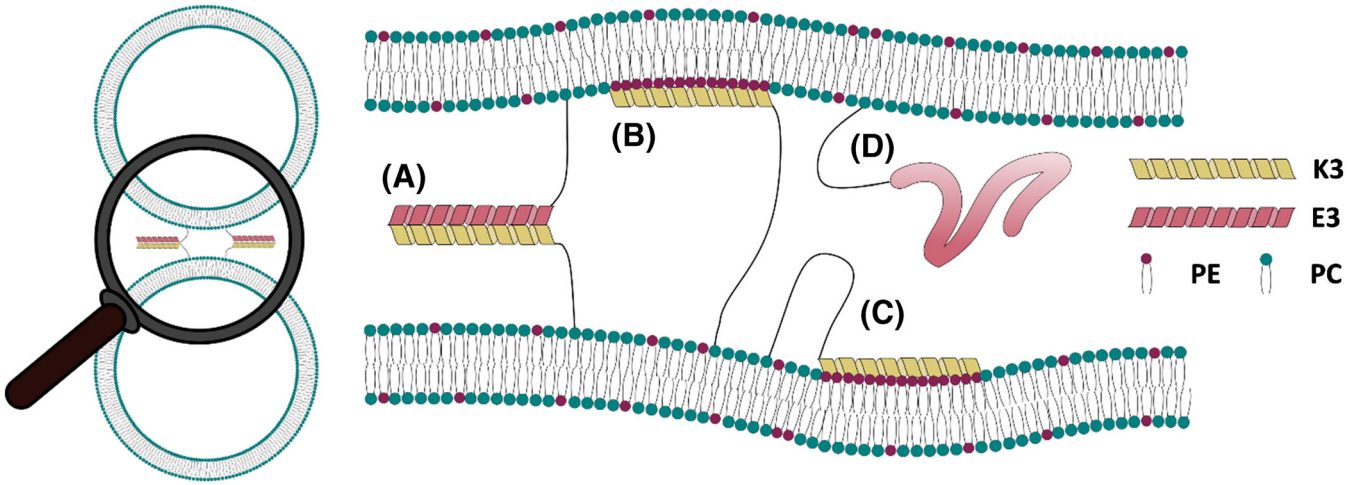


FIGURE 19 The roles of K3 peptide interactions, including formation of E3/K3 dimers, as well interaction of K3 with the membranes to induce a change in conformation and curvature. (a) Formation of a K3/E3 heterodimer, (b) K3 interaction with the opposite membrane and local PE enrichment, (c) K3 interaction with its own membrane and local PE enrichment, (d) E3 not bound to K3.[187]

the adsorbance of the positively charged K3 into the membrane surface with respect to neutral phospholipids like PC. These in silico experiments could then be confirmed by fluorescence studies.[190]

Even though the adsorption of the K3 peptide to the membrane is beneficial for initiating membrane fusion because of decreasing the activation barrier for fusion, it also means that less of the free K3 peptide is available to interact with the complementary E3 peptides. Interestingly, in the presence of peptide E3, K3 is 50 times more likely to bind to its fusion partner rather than insert itself into the phospholipid bilayer minimizing the perturbating effect of adsorption to the membrane.[190]

More recently, also other coiled-coil peptides have been considered as possible synthetic mimics of the SNARE recognition motif. In particular, the E4/K4 peptide pair was considered, which is similar to E3/K3 but contains four heptad repeats instead of three (Figure 18). In this model, the authors used cholesterol as the anchor and PEG_4 as the flexible linker and probed the propensity of the constructs to initiate the fusion of giant unilamellar vesicles (GUVs), loaded with lucigenin, with large unilamellar vesicles (LUVs) containing anionophore bis-(thioureido)decalin (Figure 20). This fusion process was monitored by fluorescence spectroscopy because the fluorescence of lucigenin was quenched by the anions delivered during the fusion process.[191] By fluorescently labeling the K4/E4 peptides, it was possible to study their interactions with one another as well as with the membrane in detail. The models showed very similar characteristics to the E3/K3 peptides. Analogously to K3, the K4 peptide shows a preference to insert itself in the lipid membrane leaving only a fraction available for interactions with the complementary peptide; E4 exclusively forms heterodimers with K4.[189] Both distribution of the E4 and K4 peptides on the membrane and the fusion process could be visualized in real time using simultaneous dual-color time-lapsed fluorescence microscopy. The same study showed that the use of nonionic surfactant Tween 20 could improve the content mixing of GUVs and LUVs indicating improved fusion efficiency. These studies showed that the K4/E4 constructs could be used as an efficient platform for drug delivery in the future.[192]

A less well-investigated system based on heterodimer forming coiled coils was designed using P2EK, P3EK, and E3/P1K coiled coils (Figure 18c), which showed higher fusion efficiency as well as more specific interactions compared with previously discussed models and thus could serve as alternative recognition motif for SNARE mimetics in the future.[193]

Other alternatives to the K3/E3-derived coiled coils were also investigated. They showed that by introducing a glutamic acid residue in position a of the *abcdefg* heptad of the peptide IZ $(IEKKIEA)_4$, it was possible to change the structure from a random coil at pH 7.0 to a coiled coil at pH 5.0. Therefore, they could use protons as an external trigger to induce structural change to an active conformation and thus membrane fusion.[194]

Although the synthetic peptide mimetics discussed up to now demonstrated that a minimalistic machinery is sufficient to mimic SNARE-induced membrane fusion, it becomes challenging to obtain detailed mechanistic insights into the native SNARE peptide when all parts of the native system are replaced. Therefore, a synthetic peptide model that was more closely related to the native structure of the peptide was designed (Figure 21). The model consists of the E3 and K3 coiled coil as small and more easily modifiable recognition motif but exhibits the native peptide structure of the transmembrane domain and linker region. Therefore, it allowed to investigate the effect the native linker and the transmembrane domain have on the fusion efficiency using FRET assays.[178]

5.3.2 | Alternative fusion peptide models

Also, non-peptidic recognition moieties, such as DNA, have been considered to mimic the SNARE recognition motif. As such, replacing the E3/K3 coiled coils in their previously described model (vide supra) with two complimentary peptide nucleic acid (PNA) strands. In this model, the anchor and linker region of the native SNARE proteins were combined with the PNA-based recognition moiety to better

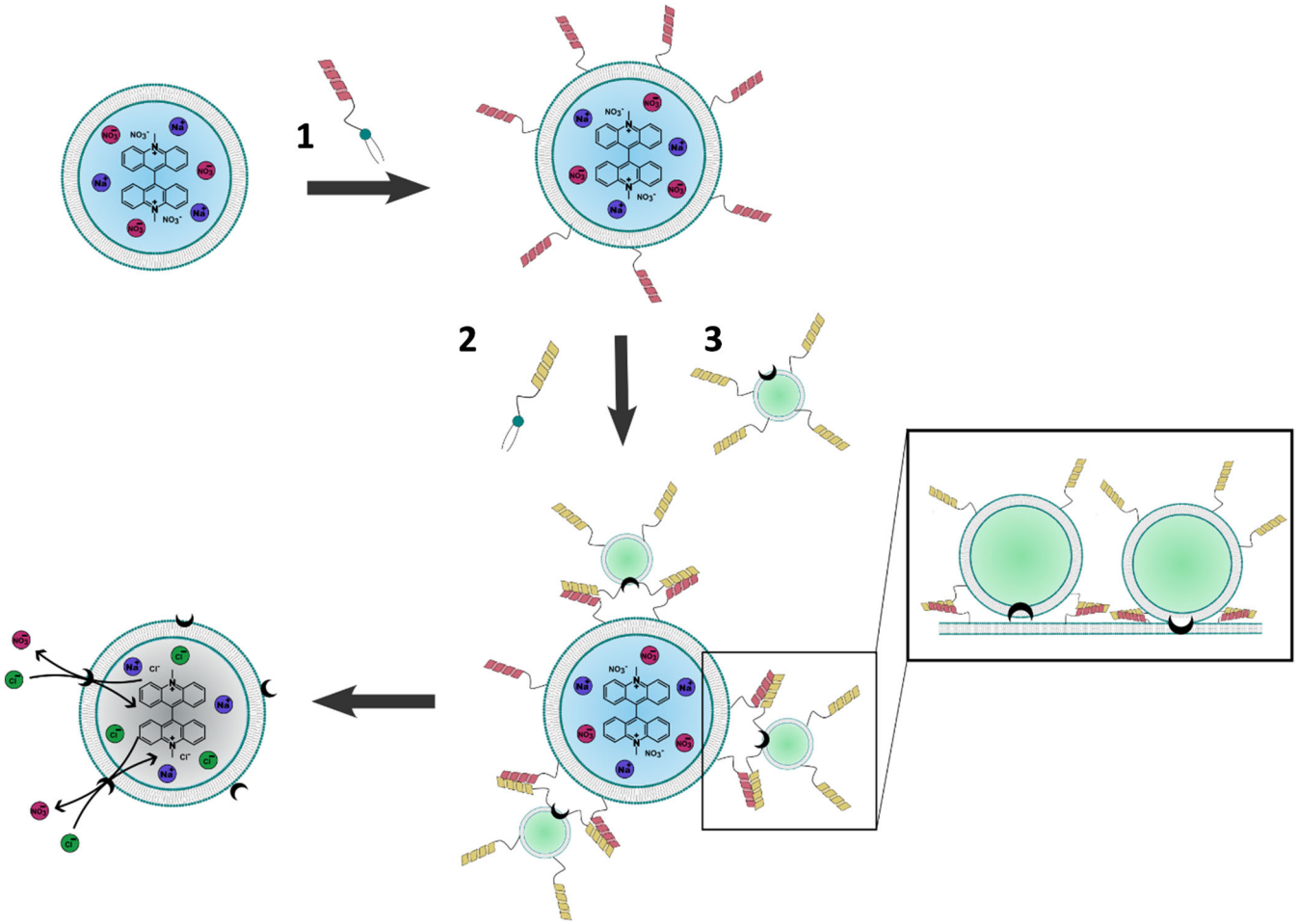


FIGURE 20 Targeted delivery of lipophilic transporter **3** via membrane fusion of vesicles incorporating peptides **1** and **2**. Final addition of NaCl to the exterior exchanges external chloride for internal nitrate, resulting in the quenching of the encapsulated lucigenin fluorophore[191]

study the natural mechanism of SNARE-induced membrane fusion. Specifically, PNAs were selected because of their specific interactions and their propensity to mimic the zippering process of the SNARE recognition motif. Only five PNA residues proved to be of sufficient length for specific recognition of the complementary strand, which is advantageous considering the often challenging synthesis of long PNA strands.[195] However, because of the stronger interactions between PNA strands compared with electrostatic interactions between coiled-coil peptides, the aggregation of the PNA achieved by a much shorter sequence was stronger.[196]

In contrast, a construct that is based on peptidic anchors was developed, whereas the fusion was induced by small molecule interactions. They utilized the propensity of Vancomycin to bind to peptides exhibiting a C-terminal D-Ala-D-Ala motif as attractive interaction between different vesicles and magainin, an AMP that can insert itself into negatively charged membranes, as anchor. The fusion process was then followed by a combination of DLS and FRET assays. The authors found that the fusion was triggered by binding to the membrane surface and the formation of dense aggregates, in which the fusion rate was very high. Interestingly, this process showed a dependency on the charge gradient between the two lipid bilayers. A surface charge difference of donor and acceptor vesicles was needed for the fusion process, whereas during fusion, this gradient decreases, leading to reduced fusion efficiency.[197]

6 | MISCELLANEOUS

As seen in the previous sections of this review, peptides are able to interact with the plasma membrane in a variety of modes. The largest classes of membrane-interactive peptides, namely, CPPs, AMPs, and fusogenic peptides (FPs), were discussed in the previous sections. Nonetheless, peptides are able to interact with membranes also in different manners, such as the membrane-located fibril formation of amyloid peptides, or peptides interacting with specific functional membrane proteins. On a different note, this review has so far only been discussing linear α-peptides interacting with the plasma membrane. Other classes of important peptide-based structures deserve a mention as well. In particular, β-peptides and cyclic peptides have too been largely investigated as possible scaffolds for membrane-targeting compounds. A brief overview over these four classes will be given in the following.

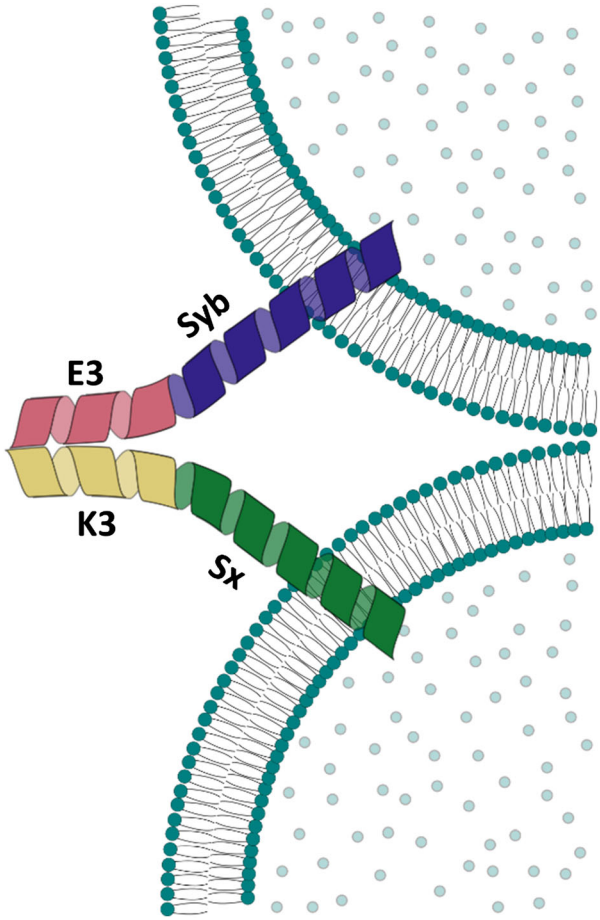


FIGURE 21 SNARE protein mimic using the structure of the native syntaxin (Sx) and synaptobrevin (Syb) transmembrane domain and linker regions with the E3/K3 coiled coils as the recognition motif.[178]

6.1 | Amyloid peptides

Amyloids are aggregates of proteins or peptides that form on the cell surface. They exhibit a β-sheet secondary structure and possess a fibrillar, non-branched morphology.[198] For years, amyloids in the human body, such as the natural exponents amylin,[199] amyloid-β peptide,[200] and α-synuclein,[201] have only been linked to the development of pathologies such as Parkinson's disease, Alzheimer's disease, or amyloidosis.[202] However, the recent discovery of functional amyloids exhibiting beneficial properties has changed the perspective on these compounds drastically. In particular, it appears they could have therapeutic interactions with human pathogens. As an example, the Alzheimer's disease correlated amyloid-β peptide (Aβ) has been shown to have a protective effect against brain infections derived from herpes viruses.[203] Aβ is in fact able to interact with the glycoproteins of the virus, which induce Aβ amyloid formation with subsequent coating and isolation of the viral particles.

After confirmation that natural amyloid structures could indeed demonstrate pharmaceutically relevant activity, the field of synthetic amyloid-like peptides developed. The intrinsic characteristics of amyloids, such as their ability to self-assemble and to form supramolecular structures, make them interesting targets for biological and medical studies. Peptides mimicking natural amyloids can readily be synthesized via solid-phase peptide synthesis and thus be employed for such applications.[204] These artificial amyloids have already found multiple fields of application, with compounds proposed as antiviral[205,206] or anticancer scaffolds,[207,208] vaccines,[209–211] or bioimaging tools.[212]

6.2 | Protein-interacting peptides

Using peptides instead of small molecules as protein ligands is advantageous as their larger size allows them to easily form highly specific interactions with their targets.[213] Because of this feature, a vast number of peptide-based drugs have been developed in the last years. Despite challenges with cellular internalization, which partly limits the targeting possibilities to membrane proteins, a significant number of protein-interacting peptides with different therapeutical uses have been successfully synthesized. A recent example is the mutant peptide R7W-MP, which targets L-type Ca^{2+} channels (LTCCs) on the membrane of cardiac cells in patients affected by Brugada syndrome.[214] This novel peptidic drug restores the function of LTCCs by interacting with their chaperone β-subunit. Ultimately, this interaction determines regulation of cell distribution of LTCCs and alteration in the life cycle of pathological mutations of the protein and therefore acts as a therapeutic agent.

The largest protein family to be targeted with peptidic drugs are the G protein-coupled receptors (GPCRs). This class of transmembrane proteins exhibits an extracellular receptor moiety that can detect molecules in the extracellular environment and activate an intracellular response. A wide variety of stimuli including ligands, such as lipids, peptides, proteins, neurotransmitters, or light, are able to trigger a response in GPCRs.[215] Already approximately 50 peptide drugs targeting GPCRs are commercially available, and a variety of new compounds are under investigation in order to produce more.[216] Semaglutide and liraglutide, for instance, are GPCR-targeting peptides acting as a glucagon-like peptide 1 receptor agonists and employed in the treatment of type-2 diabetes and as weight management drugs.[217] These two compounds, mimicking the action of the human incretin glucagon-like peptide 1, can inhibit the production of glucagon and promote the production of insulin, determining a lowering of blood sugar level.

6.3 | β-Peptides

β-Peptides differ from their α-analogs because of an extra CH_2 in the backbone between the COOH and the NH_2 functionality. This simple modification determines two important characteristics of β-peptides. On the one hand, they are able to form helical structures starting from 6-residue chains, whereas for α-peptides, usually a minimum of 10 residues is required. On the other hand, the structural differences from

α-peptides make them resistant against standard proteases and are therefore metabolically more stable.[218] Because of this biologically relevant characteristic, β-peptides have been investigated as possible alternatives for α-peptides for different applications.

Numerous in vitro studies showed that β-peptides are able to interact with the plasma membrane and form transmembrane domains.[219,220] This characteristic has then been recently exploited to design molecular rulers. β3-peptides P1 and P2 were synthesized, labelled with an Atto643 dye, and incorporated in a lipid bilayer. Subsequently, GIET spectroscopy analyses were performed in order to evaluate the distance of the dye from the lipid surface. Potential application for structures like these is the positioning of probes at given distances from the plasma membrane, in order to measure the ion concentration in its proximity.[221]

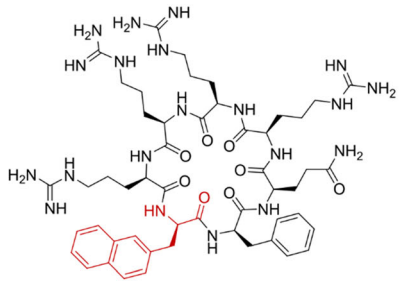
Analogously to conventional α-CPPs, β-peptides and α/β-hybrids have also shown the ability to permeate the plasma membrane following both direct penetration and endocytosis.[222,223]

Moreover, the resistance of β-peptides toward degradation by proteases made them promising candidates for the development of antimicrobic peptides. Specifically, synthesizing β-peptide analogs of active yet protease-sensitive α-peptides has proved to be a promising strategy.[224,225] As an example, a 17-residue β-peptide (named β-17) was synthesized and showed analog activity to natural AMP magainin.[226] The synthetic strand showed not only higher activity than the natural one but also higher selectivity towards bacterial cells and resistance toward trypsin protease. In particular, after 2 days of incubation of β-17 with the protease, RP-HPLC analysis proved that the peptide was not cleaved. Another possibility to generate protease-resistant molecules is the synthesis of hybrid strands consisting of alternating β- and α-amino acids.[227]

6.4 | Cyclic peptides

Cyclic peptides have aroused the interest of researchers in the recent years as they have been shown to be more resistant to proteolytic degradation compared to linear peptides and serve as potent protein ligands. Specifically, their structure allows a better interaction with the active site of proteins compared with small molecules. This makes them ideal candidates to serve as platform for the development of protein-targeting drugs.

In order to maximize their potential, cyclic peptides need to be able to penetrate the plasma membrane to reach their intracellular targets. Several natural and nonnatural cyclic peptides have already been proven to have cell-penetrating abilities.[228,229] Interestingly, cyclic structures appear to facilitate not only internalization of the compounds but also endosomal escape. A family of cyclic compounds was synthesized starting from the already known cFΦR4 peptide.[230] Like their precursor, these new CPPs are formed by a cyclic arginine-rich strand characterized by an L-2-naphthylalanine residue (Φ; Figure 22).



1

N	Abbreviation	Sequence
1	cFΦR$_4$	cyclo(FΦRRRRQ)
2	cFΦR$_4^{Rho}$	cyclo(FΦRRRRQ)-K(Rho)
3	cFΦR$_4^{Dex}$	cyclo(FΦRRRRQ)-K(Dex)
4	cFΦR$_4$FITC	cyclo(FΦRRRRQ)-K(FITC)
5	cFΦR$_4$-R5	cyclo(FΦRRRRQ)-RRRRRK(Rho)
6	cFΦR$_4$-A5	cyclo(FΦRRRRQ)-AAAAAK(Rho)
7	cFΦR$_4$-F4	cyclo(FΦRRRRQ)-FFFF-K(Rho)
8	cFΦR$_4$-PCP	cyclo(FΦRRRRQ)-miniPEGDE(pCAP)LI
9	bicyclo(FΦR$_4$-A5)^{Rho}	[Tm(AAAAA)K(RRRRΦF)J]K(Rho)
10	bicyclo(FΦR$_4$-A7)^{Rho}	[Tm(AAAAAAA)K(RRRRΦF)J]K(Rho)
11	bicyclo(FΦR$_4$-RARAR)^{Rho}	[Tm(RARAR)K(RRRRΦF)J]K(Rho)
12	bicyclo(FΦR$_4$-DADAD)^{Rho}	[Tm(DADAD)K(RRRRΦF)J]K(Rho)
13	monocyclo(FΦR$_4$-A5)^{Rho}	cyclo(AAAAARRRRΦF)-K(Rho)
14	monocyclo(FΦR$_4$-A7) ^{Rho}	cyclo(AAAAAAARRRRΦF)K(Rho)

FIGURE 22 cFΦR4 structure with the 2-naphthylalanine residue highlighted in red. The table reports the structures of the synthesized analogs.

Flow cytometry analysis was performed on the fluorescent-labeled peptides and showed that they exhibit a high endosomal escape activity through vesicle budding formation in the endosomal membrane.[100] Some cyclic peptides, like WR_4 and WR_5, two short cyclic peptides synthesized with L-amino acids and rich in charged residues, have also shown endocytic-independent internalization ability.[231]

The interest in cyclic peptides has also revolved around their possible use as antimicrobial agents. Natural compounds, such as tyrocidines, have shown antibacterial activity against the malaria-responsible parasite *Plasmodium falciparum*, by inhibiting their growth and thus blocking their life cycle.[232] Also, synthetic compounds have shown similar properties. For instance, short cyclic peptides with an even number of alternating D,L-α-amino acids exhibit high activity against *Staphylococcus aureus* and other gram-positive bacteria.[233]

7 | CONCLUSION AND OUTLOOK

In this review, we saw how specific peptide–membrane interactions can be found in naturally occurring peptides with cell-penetrating, antimicrobial, or fusogenic activity. These compounds have aroused the interest of researchers because of their biological activity and their potential to the development of synthetic derivatives in a highly programmable manner. These analogues aim to possess higher activity while exhibiting lower toxicity with respect to their natural counterparts.

CPPs are a promising tool for the delivery of biologically active molecules inside live cells. In particular, peptidic structures able to transport cargos are extremely interesting because of their biological compatibility. New natural peptides with cell-penetrating capabilities keep being discovered, whereas synthetic analogues are developed in order to generate compounds with improved drug-like properties. These derivatives are able to cross the plasma membrane of eukaryotic cells without inducing cell death and therefore can vehiculate active molecules inside the cytosol without damage rendering them great candidates for the development of drug delivery systems. Even though CPPs are known to be able to cross the membrane through different mechanisms, a full understanding is still missing. Synthetically modified peptides provide the opportunity to close this gap by providing insights into structure–activity relationships.

AMPs, on the other hand, are a promising alternative to classical antibiotics providing a potential solution for the antibiotic crisis. Specifically, AMPs avoid the formation of resistance in bacteria by interacting with the cell membrane directly. These peptides either disrupt the membrane through pore formation, causing cell death through leakage, or cross the membrane to inhibit important intracellular processes. Besides their potential to replace antibiotics, they can also be used to further enhance their potency by increasing their uptake. By introducing smart, synthetic modifications, the properties of AMPs can be finetuned. For instance, introducing D-amino acids to the sequence of AMPs or a PEG unit lowers the negative effect of the hemolytic activity. Also, assembling them into nanostructures can have similar effects, while simultaneously increasing the bactericidal effect. The antimicrobial activity can be further enhanced by adding fatty acids or amidate the carboxylic end of the AMP.

Model structures of fusion peptides as well as their interactions with the membrane have been widely studied. The introduction of coiled-coil peptides anchored in the phospholipid bilayer is of central interest to study and induce membrane fusion. The E3/K3 coiled-coil model and its variants have found widespread use as SNARE peptide mimics because of the relative simplicity as well as similarity to the native recognition motif. Moreover, K3 coiled coils showed interactions with the lipid bilayer that reorganizes the membrane composition and decreases the energy barrier required for fusion. Furthermore, the effect and position of the lipid anchor as well as the membrane composition on the efficiency of the fusion have been studied. However, altering the recognition motif, for instance, to the extended E4/K4 pair was demonstrated to be a useful tool for membrane fusion mimicry. Models utilizing other than coiled-coil recognition motifs have also been explored, such as the use of PNA or small molecule interactions to induce membrane fusion. This shows the potential for greater variety in the models mimicking membrane fusion proteins.

Besides the larger groups of peptides interacting with the plasma membrane, such as CPPs, AMPs, and FPs, also, the properties of other classes have been explored. Amyloid peptides and protein-interacting peptides have shown interesting possible applications in pharmaceutical and medical fields. Furthermore, modifications such as the use of β-amino acids or cyclic peptide analogs contributed to the development of compounds with better activity profiles compared with classical α-amino acids linear strands.

Ultimately, synthetic peptides and peptide analogs are extremely promising compounds that can be employed either as analytical tools for a better understanding of human physiology or as novel therapeutic moieties.

ACKNOWLEDGMENTS

Ilze Lāce, Elena R. Cotroneo, and Nils Hesselbarth thank their late mentor Ulf Diederichsen for setting them out on their PhD journey and introducing them to the fascinating field of bioorganic and peptide chemistry. This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy (EXC 2067/1-390729940 to NAS). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

IL, ERC, NH, and NAS conceived the idea of the review. All authors searched the literature and wrote parts of the review. ERC focused on the CPP section, NH on AMPs, and IL on the fusion peptide section. All authors discussed and revised the text and agreed to the final version of the manuscript.

ORCID

Ilze Lāce https://orcid.org/0000-0002-4023-6988
Elena R. Cotroneo https://orcid.org/0000-0003-0388-8626
Nils Hesselbarth https://orcid.org/0000-0002-6820-4232
Nadja A. Simeth https://orcid.org/0000-0001-8130-883X

REFERENCES

1. Avci FG, Akbulut BS, Ozkirimli E. Membrane active peptides and their biophysical characterization. *Biomolecules*. 2018;8(3):77. doi:10.3390/biom8030077
2. Rady I, Siddiqui IA, Rady M, Mukhtar H. Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy. *Cancer Lett*. 2017;402:16-31. doi:10.1016/j.canlet.2017.05.010
3. Nascimento FD, Sancey L, Pereira A, et al. The natural cell-penetrating peptide Crotamine targets tumor tissue *in vivo* and triggers a lethal calcium-dependent pathway in cultured cells. *Mol Pharm*. 2012;9(2):211-221. doi:10.1021/mp2000605
4. McErlean EM, Ziminska M, McCrudden CM, et al. Rational design and characterisation of a linear cell penetrating peptide for non-viral gene delivery. *J Control Release*. 2021;330:1288-1299. doi:10.1016/j.jconrel.2020.11.037
5. Kauffman WB, Guha S, Wimley WC. Synthetic molecular evolution of hybrid cell penetrating peptides. *Nat Commun*. 2018;9(1):2568. doi:10.1038/s41467-018-04874-6
6. Pooga M, Hällbrink M, Zorko M, Langel Ü. Cell penetration by transportan. *FASEB j*. 1998;12(1):67-77. doi:10.1096/fsb2fasebj.12.1.67
7. Schmidt N, Mishra A, Lai GH, Wong GCL. Arginine-rich cell-penetrating peptides. *FEBS Lett*. 2010;584(9):1806-1813. doi:10.1016/j.febslet.2009.11.046
8. Bahar A, Ren D. Antimicrobial peptides. *Pharmaceuticals*. 2013;6(12):1543-1575. doi:10.3390/ph6121543
9. Maturana P, Gonçalves S, Martinez M, et al. Interactions of "de novo" designed peptides with bacterial membranes: implications in the antimicrobial activity. *Biochim Biophys Acta - Biomembranes*. 2020;1862(11):183443. doi:10.1016/j.bbamem.2020.183443
10. Ventura CR, Wiedman GR. Substituting azobenzene for proline in melittin to create photomelittin: a light-controlled membrane active peptide. *Biochim Biophys Acta - Biomembranes*. 2021;1863(12):183759. doi:10.1016/j.bbamem.2021.183759
11. Jiang Z, Vasil AI, Hale JD, Hancock REW, Vasil ML, Hodges RS. Effects of net charge and the number of positively charged residues on the biological activity of amphipathic α-helical cationic antimicrobial peptides. *Biopolymers*. 2008;90(3):369-383. doi:10.1002/bip.20911
12. Oren Z, Shai Y. A class of highly potent antibacterial peptides derived from pardaxin, a pore-forming peptide isolated from Moses sole fish Pardachirus marmoratus. *Eur J Biochem*. 1996;237(1):303-310. doi:10.1111/j.1432-1033.1996.0303n.x
13. Jia F, Wang J, Peng J, et al. D-Amino acid substitution enhances the stability of antimicrobial peptide polybia-CP. *ABBS*. 2017;49(10):916-925. doi:10.1093/abbs/gmx091
14. White JM, Delos SE, Brecher M, Schornberg K. Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. *Crit Rev Biochem Mol Biol*. 2008;43(3):189-219. doi:10.1080/10409230802058320
15. Mayer A. Membrane fusion in eukaryotic cells. *Annu Rev Cell Dev Biol*. 2002;18(1):289-314. doi:10.1146/annurev.cellbio.18.032202.114809
16. Vance TDR, Lee JE. Virus and eukaryote fusogen superfamilies. *Curr Biol*. 2020;30(13):R750-R754. doi:10.1016/j.cub.2020.05.029
17. Han J, Pluhackova K, Böckmann RA. The multifaceted role of SNARE proteins in membrane fusion. *Front Physiol*. 2017;8:8. doi:10.3389/fphys.2017.00005
18. Ungar D, Hughson FM. SNARE protein structure and function. *Annu Rev Cell Dev Biol*. 2003;19(1):493-517. doi:10.1146/annurev.cellbio.19.110701.155609
19. Hanson JM, Gettel DL, Tabaei SR, et al. Cholesterol-enriched domain formation induced by viral-encoded, membrane-active amphipathic peptide. *Biophys J*. 2016;110(1):176-187. doi:10.1016/j.bpj.2015.11.032
20. Grage SL, Afonin S, Kara S, Buth G, Ulrich AS. Membrane thinning and thickening induced by membrane-active amphipathic peptides. *Front Cell Dev Biol*. 2016;4:4. doi:10.3389/fcell.2016.00065
21. Behzadipour, Hemmati. Considerations on the rational design of covalently conjugated cell-penetrating peptides (CPPs) for intracellular delivery of proteins: a guide to CPP selection using glucarpidase as the model cargo molecule. *Molecules*. 2019;24(23):4318. doi:10.3390/molecules24234318
22. Lichtenstein M, Zabit S, Hauser N, et al. TAT for enzyme/protein delivery to restore or destroy cell activity in human diseases. *Life*. 2021;11(9):924. doi:10.3390/life11090924
23. Belnoue E, Leystra AA, Carboni S, et al. Novel protein-based vaccine against self-antigen reduces the formation of sporadic colon adenomas in mice. *Cancer*. 2021;13(4):845. doi:10.3390/cancers13040845
24. Wang Y, Liu C-H, Ji T, et al. Intravenous treatment of choroidal neovascularization by photo-targeted nanoparticles. *Nat Commun*. 2019;10(1):804. doi:10.1038/s41467-019-08690-4
25. Silva S, Almeida A, Vale N. Combination of cell-penetrating peptides with nanoparticles for therapeutic application: a review. *Biomolecules*. 2019;9(1):22. doi:10.3390/biom9010022
26. Frankel A, Carl P. Cellular uptake of the tat protein from human immunodeficiency virus. *Cell*. 1988;55(6):1189-1193. doi:10.1016/0092-8674(88)90263-2
27. Derossi D, Joliot AH, Chassaing G, Prochiantz A. The third helix of the antennapedia homeodomain translocates through biological membranes. *J Biol Chem*. 1994;269(14):10444-10450. doi:10.1016/S0021-9258(17)34080-2
28. Lulla RR, Goldman S, Yamada T, et al. Phase I trial of P28 (NSC745104), a non-HDM2-mediated peptide inhibitor of P53 ubiquitination in pediatric patients with recurrent or progressive central nervous system tumors: a pediatric brain tumor consortium study. *NEUONC*. 2016;18(9):1319-1325. doi:10.1093/neuonc/now047
29. Liu BR, Huang Y-W, Aronstam RS, Lee H-J. Comparative mechanisms of protein transduction mediated by cell-penetrating peptides in prokaryotes. *J Membr Biol*. 2015;248(2):355-368. doi:10.1007/s00232-015-9777-x
30. Lee H-M, Ren J, Tran KM, et al. Identification of efficient prokaryotic cell-penetrating peptides with applications in bacterial biotechnology. *Commun Biol*. 2021;4(1):205. doi:10.1038/s42003-021-01726-w
31. Ochocki JD, Mullen DG, Wattenberg EV, Distefano MD. Evaluation of a cell penetrating prenylated peptide lacking an intrinsic fluorophore via in situ click reaction. *Bioorg Med Chem Lett*. 2011;21(17):4998-5001. doi:10.1016/j.bmcl.2011.04.138
32. Gao S, Simon MJ, Hue CD, Morrison B, Banta S. An unusual cell penetrating peptide identified using a plasmid display-based functional selection platform. *ACS Chem Biol*. 2011;6(5):484-491. doi:10.1021/cb100423u
33. Bernal F, Tyler AF, Korsmeyer SJ, Walensky LD, Verdine GL. Reactivation of the P53 tumor suppressor pathway by a stapled P53 peptide. *J Am Chem Soc*. 2007;129(9):2456-2457. doi:10.1021/ja0693587
34. Ochocki JD, Igbavboa U, Wood WG, Arriaga EA, Wattenberg EV, Distefano MD. Evaluation of prenylated peptides for use in cellular imaging and biochemical analysis. In: Nixon AE, ed. *Therapeutic Peptides*. Methods in Molecular Biology. Vol.1088. Totowa, NJ: Humana Press; 2014:213-223. doi:10.1007/978-1-62703-673-3_14

35. Covic L, Gresser AL, Talavera J, Swift S, Kuliopulos A. Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. *Proc Natl Acad Sci U S A*. 2002;99(2):643-648. doi:10.1073/pnas.022460899
36. Futaki S, Suzuki T, Ohashi W, et al. Arginine-rich peptides. *J Biol Chem*. 2001;276(8):5836-5840. doi:10.1074/jbc.M007540200
37. Alizadeh S, Irani S, Bolhassani A, Sadat SM. HR9: an important cell penetrating peptide for delivery of HCV NS3 DNA into HEK-293T cells. *Avicenna J Med Biotechnol*. 2020;12(1):8.
38. Mai JC, Shen H, Watkins SC, Cheng T, Robbins PD. Efficiency of protein transduction is cell type-dependent and is enhanced by dextran sulfate. *J Biol Chem*. 2002;277(33):30208-30218. doi:10.1074/jbc.M204202200
39. Porosk L, Arukuusk P, Põhako K, et al. Enhancement of SiRNA transfection by the optimization of fatty acid length and histidine content in the CPP. *Biomater Sci*. 2019;7(10):4363-4374. doi:10.1039/C9BM00688E
40. Tünnemann G, Ter-Avetisyan G, Martin RM, Stöckl M, Herrmann A, Cardoso MC. Live-cell analysis of cell penetration ability and toxicity of oligo-arginines. *J Pept Sci*. 2008;14(4):469-476. doi:10.1002/psc.968
41. Liu BR, Lo S-Y, Liu C-C, et al. Endocytic trafficking of nanoparticles delivered by cell-penetrating peptides comprised of nona-arginine and a penetration accelerating sequence. *PLoS ONE*. 2013;8(6):e67100. doi:10.1371/journal.pone.0067100
42. Liu BR, Liou J-S, Chen Y-J, Huang Y-W, Lee H-J. Delivery of nucleic acids, proteins, and nanoparticles by arginine-rich cell-penetrating peptides in rotifers. *Marine Biotechnol*. 2013;15(5):584-595. doi:10.1007/s10126-013-9509-0
43. Choi DH, Park YS. Arginine-rich peptide coated PLGA nanoparticles enhance polymeric delivery of antisense HIF1α-oligonucleotide to fully differentiated stiff adipocytes. *Toxicol Environ Health Sci*. 2019;11(1):1-10. doi:10.1007/s13530-019-0382-8
44. Zhang C, Ren W, Liu Q, Tan Z, Li J, Tong C. Transportan-derived cell-penetrating peptide delivers SiRNA to inhibit replication of influenza virus in vivo. *DDDT*. 2019;13:1059-1068. doi:10.2147/DDDT.S195481
45. Soomets U, Lindgren M, Gallet X, et al. Deletion analogues of transportan. *Biochim Biophys Acta*. 12:165-176.
46. Ezzat K, el Andaloussi S, Zaghloul EM, et al. PepFect 14, a novel cell-penetrating peptide for oligonucleotide delivery in solution and as solid formulation. *Nucleic Acids Res*. 2011;39(12):5284-5298. doi:10.1093/nar/gkr072
47. Freimann K, Arukuusk P, Kurrikoff K, et al. Optimization of in vivo DNA delivery with NickFect peptide vectors. *J Control Release*. 2016;241:135-143. doi:10.1016/j.jconrel.2016.09.022
48. Arukuusk P, Pärnaste L, Hällbrink M, Langel Ü. PepFects and NickFects for the intracellular delivery of nucleic acids. In: Langel Ü, ed. *Cell-Penetrating Peptides*. Methods in Molecular Biology. Vol.1324. New York, NY: Springer New York; 2015:303-315. doi:10.1007/978-1-4939-2806-4_19
49. Falanga A, Lombardi L, Tarallo R, et al. The intriguing journey of GH625-dendrimers. *RSC Adv*. 2017;7(15):9106-9114. doi:10.1039/C6RA28405A
50. Galdiero S, Falanga A, Morelli G, Galdiero M. GH625: a milestone in understanding the many roles of membranotropic peptides. *Biochim Biophys Acta - Biomembranes*. 2015;1848(1):16-25. doi:10.1016/j.bbamem.2014.10.006
51. Bendifallah N, Rasmussen FW, Zachar V, Ebbesen P, Nielsen PE, Koppelhus U. Evaluation of cell-penetrating peptides (CPPs) as vehicles for intracellular delivery of antisense peptide nucleic acid (PNA). *Bioconjug Chem*. 2006;17(3):750-758. doi:10.1021/bc050283q
52. Song J, Qian Z, Sahni A, Chen K, Pei D. Cyclic cell-penetrating peptides with single hydrophobic groups. *ChemBioChem*. 2019;20(16):2085-2088. doi:10.1002/cbic.201900370
53. Kosuge. *Cellular Internalization and Distribution of Arginine-Rich Peptides as a Function of Extracellular Peptide Concentration*. Serum, and Plasma Membrane Associated Proteoglycans.
54. Nomura K, Kawano K, Kawaguchi Y, et al. Hemopexin as a potential binding partner of arginine-rich cell-penetrating peptides in serum. *ACS Pharmacol Transl Sci*. 2022;5(8):603-615. doi:10.1021/acsptsci.2c00043
55. Hirose H, Takeuchi T, Osakada H, et al. Transient focal membrane deformation induced by arginine-rich peptides leads to their direct penetration into cells. *Mol Ther*. 2012;20(5):984-993. doi:10.1038/mt.2011.313
56. Lee H-J, Huang Y-W, Aronstam RS. Intracellular delivery of nanoparticles mediated by lactoferricin cell-penetrating peptides in an endocytic pathway. *J Nanosci Nanotechnol*. 2019;9:613-621.
57. Moghal MMR, Islam MZ, Hossain F, Saha SK, Yamazaki M. Role of membrane potential on entry of cell-penetrating peptide transportan 10 into single vesicles. *Biophys J*. 2020;118(1):57-69. doi:10.1016/j.bpj.2019.11.012
58. Lin C-C, Bachmann M, Bachler S, Venkatesan K, Dittrich PS. Tunable membrane potential reconstituted in giant vesicles promotes permeation of cationic peptides at nanomolar concentrations. *ACS Appl Mater Interfaces*. 2018;10(49):41909-41916. doi:10.1021/acsami.8b12217
59. Gao X, Hong S, Liu Z, Yue T, Dobnikar J, Zhang X. Membrane potential drives direct translocation of cell-penetrating peptides. *Nanoscale*. 2019;11(4):1949-1958. doi:10.1039/C8NR10447F
60. Allolio C, Magarkar A, Jurkiewicz P, et al. Arginine-rich cell-penetrating peptides induce membrane multilamellarity and subsequently enter via formation of a fusion pore. *Proc Natl Acad Sci U S A*. 2018;115(47):11923-11928. doi:10.1073/pnas.1811520115
61. Thennarasu S, Tan A, Penumatchu R, Shelburne CE, Heyl DL, Ramamoorthy A. Antimicrobial and membrane disrupting activities of a peptide derived from the human cathelicidin antimicrobial peptide LL37. *Biophys J*. 2010;98(2):248-257. doi:10.1016/j.bpj.2009.09.060
62. Direct translocation of cell penetrating peptides through membranes occurs via the formation of inverted micelles. *J Pept Sci*. 2014;20:S33-S39. doi:10.1002/psc.2686
63. Deshayes S, Morris M, Heitz F, Divita G. Delivery of proteins and nucleic acids using a non-covalent peptide-based strategy. *Adv Drug Deliv Rev*. 2008;60(4–5):537-547. doi:10.1016/j.addr.2007.09.005
64. Qian S, Wang W, Yang L, Huang HW. Structure of the alamethicin pore reconstructed by X-ray diffraction analysis. *Biophys J*. 2008;94(9):3512-3522. doi:10.1529/biophysj.107.126474
65. Leveritt JM, Pino-Angeles A, Lazaridis T. The structure of a melittin-stabilized pore. *Biophys J*. 2015;108(10):2424-2426. doi:10.1016/j.bpj.2015.04.006
66. Hasan M, Karal MAS, Levadnyy V, Yamazaki M. Mechanism of initial stage of pore formation induced by antimicrobial peptide magainin 2. *Langmuir*. 2018;34(10):3349-3362. doi:10.1021/acs.langmuir.7b04219
67. Pouny Y, Rapaport D, Mor A, Nicolas P, Shai Y. Interaction of antimicrobial dermaseptin and its fluorescently labeled analogs with phospholipid membranes. *Biochemistry*. 1992;31(49):12416-12423. doi:10.1021/bi00164a017
68. Zamora-Carreras H, Strandberg E, Mühlhäuser P, et al. Alanine scan and 2H NMR analysis of the membrane-active peptide BP100 point to a distinct carpet mechanism of action. *Biochim Biophys Acta*. 1858;11:1328-1338.
69. Kawamoto S, Miyakawa T, Takasu M, et al. Cell-penetrating peptide induces various deformations of lipid bilayer membrane: inverted micelle, double bilayer, and transmembrane. *Int J Quant Chem*. 2012;112(1):178-183. doi:10.1002/qua.23177
70. Kawamoto S, Takasu M, Miyakawa T, et al. Inverted micelle formation of cell-penetrating peptide studied by coarse-grained

simulation: importance of attractive force between cell-penetrating peptides and lipid head group. *J Chem Phys*. 2011;134(9):095103. doi:10.1063/1.3555531
71. Khandia R, Munjal A, Kumar A, Singh G, Karthik K, Dhama K. Cell penetrating peptides: biomedical/therapeutic applications with emphasis as promising futuristic hope for treating cancer. *Int J Pharm*. 2017;13(7):677-689. doi:10.3923/ijp.2017.677.689
72. Richard JP, Melikov K, Vives E, et al. Cell-penetrating peptides. *J Biol Chem*. 2003;278(1):585-590. doi:10.1074/jbc.M209548200
73. Fretz MM, Penning NA, Al-Taei S, et al. Temperature-, concentration- and cholesterol-dependent translocation of L- and D-octa-arginine across the plasma and nuclear membrane of CD34+ leukaemia cells. *Biochem J*. 2007;403(2):335-342. doi:10.1042/BJ20061808
74. Hymel HC, Rahnama A, Sanchez OM, Liu D, Gauthier TJ, Melvin AT. How cargo identity alters the uptake of cell-penetrating peptide (CPP)/cargo complexes: a study on the effect of net cargo charge and length. *Cell*. 2022;11(7):1195. doi:10.3390/cells11071195
75. Lorents A, Säälik P, Langel Ü, Pooga M. Arginine-rich cell-penetrating peptides require nucleolin and cholesterol-poor subdomains for translocation across membranes. *Bioconjug Chem*. 2018;29(4):1168-1177. doi:10.1021/acs.bioconjchem.7b00805
76. Murayama T, Masuda T, Afonin S, et al. Loosening of lipid packing promotes oligoarginine entry into cells. *Angew Chem Int Ed*. 2017;56(26):7644-7647. doi:10.1002/anie.201703578
77. Pae J, Säälik P, Liivamägi L, et al. Translocation of cell-penetrating peptides across the plasma membrane is controlled by cholesterol and microenvironment created by membranous proteins. *J Control Release*. 2014;192:103-113. doi:10.1016/j.jconrel.2014.07.002
78. Almeida C, Maniti O, Di Pisa M, Swiecicki J-M, Ayala-Sanmartin J. Cholesterol re-organisation and lipid de-packing by arginine-rich cell penetrating peptides: role in membrane translocation. *PLoS ONE*. 2019;14(1):e0210985. doi:10.1371/journal.pone.0210985
79. Arsov Z, Nemec M, Schara M, Johansson H, Langel Ü, Zorko M. Cholesterol prevents interaction of the cell-penetrating peptide transportan with model lipid membranes. *J Pept Sci*. 2008;14(12):1303-1308. doi:10.1002/psc.1062
80. Guo R-C, Zhang X-H, Fan P-S, et al. In vivo self-assembly induced cell membrane phase separation for improved peptide drug internalization. *Angew Chem*. 2021;60(47):25128-25134. doi:10.1002/anie.202111839
81. Doherty GJ, McMahon HT. *Mechanisms of Endocytosis*; 2009:69.
82. Hu G, Zheng W, Li A, et al. A novel CAV derived cell-penetrating peptide efficiently delivers exogenous molecules through caveolae-mediated endocytosis. *Vet Res*. 2018;49(1):16. doi:10.1186/s13567-018-0513-2
83. Magzoub M, Sandgren S, Lundberg P, et al. N-terminal peptides from unprocessed prion proteins enter cells by macropinocytosis. *Biochem Biophys Res Commun*. 2006;348(2):379-385. doi:10.1016/j.bbrc.2006.07.065
84. Futaki S, Nakase I. Cell-surface interactions on arginine-rich cell-penetrating peptides allow for multiplex modes of internalization. *Acc Chem Res*. 2017;50(10):2449-2456. doi:10.1021/acs.accounts.7b00221
85. Kerr MC, Teasdale RD. Defining macropinocytosis. *Traffic*. 2009;10(4):364-371. doi:10.1111/j.1600-0854.2009.00878.x
86. Richard JP, Melikov K, Brooks H, Prevot P, Lebleu B, Chernomordik LV. Cellular uptake of unconjugated TAT peptide involves clathrin-dependent endocytosis and heparan sulfate receptors. *J Biol Chem*. 2005;280(15):15300-15306. doi:10.1074/jbc.M401604200
87. Tanaka G, Nakase I, Fukuda Y, et al. CXCR4 stimulates macropinocytosis: implications for cellular uptake of arginine-rich cell-penetrating peptides and HIV. *Chem Biol*. 2012;19(11):1437-1446. doi:10.1016/j.chembiol.2012.09.011
88. Nakase I, Niwa M, Takeuchi T, et al. Cellular uptake of arginine-rich peptides: roles for macropinocytosis and actin rearrangement. *Mol Ther*. 2004;10(6):1011-1022. doi:10.1016/j.ymthe.2004.08.010
89. Arukuusk P, Pärnaste L, Margus H, et al. Differential endosomal pathways for radically modified peptide vectors. *Bioconjug Chem*. 2013;24(10):1721-1732. doi:10.1021/bc4002757
90. Kawaguchi Y, Takeuchi T, Kuwata K, et al. Syndecan-4 is a receptor for clathrin-mediated endocytosis of arginine-rich cell-penetrating peptides. *Bioconjug Chem*. 2016;27(4):1119-1130. doi:10.1021/acs.bioconjchem.6b00082
91. Ferrari A, Pellegrini V, Arcangeli C, Fittipaldi A, Giacca M, Beltram F. Caveolae-mediated internalization of extracellular HIV-1 tat fusion proteins visualized in real time. *Mol Ther*. 2003;8(2):284-294. doi:10.1016/S1525-0016(03)00122-9
92. Säälik P, Padari K, Niinep A, et al. Protein delivery with transportans is mediated by caveolae rather than flotillin-dependent pathways. *Bioconjug Chem*. 2009;20(5):877-887. doi:10.1021/bc800416f
93. Veiman K-L, Mäger I, Ezzat K, et al. PepFect14 peptide vector for efficient gene delivery in cell cultures. *Mol Pharm*. 2013;10(1):199-210. doi:10.1021/mp3003557
94. Ye J, Pei X, Cui H, et al. Cellular uptake mechanism and comparative in vitro cytotoxicity studies of monomeric LMWP-SiRNA conjugate. *J Ind Eng Chem*. 2018;63:103-111. doi:10.1016/j.jiec.2018.02.005
95. Kondow-McConaghy HM, Muthukrishnan N, Erazo-Oliveras A, Najjar K, Juliano RL, Pellois J-P. Impact of the endosomal escape activity of cell-penetrating peptides on the endocytic pathway. *ACS Chem Biol*. 2020;15(9):2355-2363. doi:10.1021/acschembio.0c00319
96. Smith SA, Selby LI, Johnston APR, Such GK. The endosomal escape of nanoparticles: toward more efficient cellular delivery. *Bioconjug Chem*. 2019;10:263-272.
97. Chu D. *Rational Modification of Oligoarginine for Highly Efficient SiRNA Delivery: Structure-Activity Relationship and Mechanism of Intracellular Trafficking of SiRNA*:36.
98. Plaza-GA I, Manzaneda-González V, Kisovec M, et al. PH-triggered endosomal escape of pore-forming Listeriolysin O toxin-coated gold nanoparticles. *J Nanobiotechnol*. 2019;17(1):108. doi:10.1186/s12951-019-0543-6
99. Appelbaum JS. *Arginine Topology Controls Escape of Minimally Cationic Proteins from Early Endosomes to the Cytoplasm*:12.
100. Qian Z, Martyna A, Hard RL, et al. Discovery and mechanism of highly efficient cyclic cell-penetrating peptides. *Biochemistry*. 2016;55(18):2601-2612. doi:10.1021/acs.biochem.6b00226
101. Tamemoto N, Akishiba M, Sakamoto K, Kawano K, Noguchi H, Futaki S. Rational design principles of attenuated cationic lytic peptides for intracellular delivery of biomacromolecules. *Mol Pharm*. 2020;17(6):2175-2185. doi:10.1021/acs.molpharmaceut.0c00312
102. Azuma Y, Imai H, Kawaguchi Y, Nakase I, Kimura H, Futaki S. Modular redesign of a cationic lytic peptide to promote the endosomal escape of biomacromolecules. *Angew Chem Int Ed*. 2018;57(39):12771-12774. doi:10.1002/anie.201807534
103. Masuda T, Hirose H, Baba K, et al. An artificial amphiphilic peptide promotes endocytic uptake by inducing membrane curvature. *Bioconjug Chem*. 2020;31(6):1611-1615. doi:10.1021/acs.bioconjchem.0c00239
104. Sahni A, Qian Z, Pei D. Cell-penetrating peptides escape the endosome by inducing vesicle budding and collapse. *ACS Chem Biol*. 2020;15(9):2485-2492. doi:10.1021/acschembio.0c00478
105. Manteghi R, Pallagi E, Olajos G, Csóka I. Pegylation and formulation strategy of anti-microbial peptide (AMP) according to the quality by design approach. *Eur J Pharm Sci*. 2020;144:105197. doi:10.1016/j.ejps.2019.105197
106. Riedl S, Zweytick D, Lohner K. Membrane-active host defense peptides—challenges and perspectives for the development of novel

anticancer drugs. *Chem Phys Lipids*. 2011;164(8):766-781. doi:10.1016/j.chemphyslip.2011.09.004
107. Bowdish D, Davidson D, Hancock R. A re-evaluation of the role of host defence peptides in mammalian immunity. *CPPS*. 2005;6(1):35-51. doi:10.2174/1389203053027494
108. Zasloff M. Antimicrobial peptides in health and disease. *N Engl J Med*. 2002;347(15):1199-1200. doi:10.1056/NEJMe020106
109. Nguyen LT, Haney EF, Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol*. 2011;29(9):464-472. doi:10.1016/j.tibtech.2011.05.001
110. Zhang L, Gallo RL. Antimicrobial peptides. *Curr Biol*. 2016;26(1):R14-R19. doi:10.1016/j.cub.2015.11.017
111. Loeffler JM, Nelson D, Fischetti VA. Rapid killing of *streptococcus pneumoniae* with a bacteriophage cell wall hydrolase. *Science*. 2001;294(5549):2170-2172. doi:10.1126/science.1066869
112. Brumfitt W. Nisin, alone and combined with peptidoglycan-modulating antibiotics: activity against methicillin-resistant staphylococcus aureus and vancomycin-resistant enterococci. *J Antimicrob Chemother*. 2002;50(5):731-734. doi:10.1093/jac/dkf190
113. Naghmouchi K, Le Lay C, Baah J, Drider D. Antibiotic and antimicrobial peptide combinations: synergistic inhibition of pseudomonas fluorescens and antibiotic-resistant variants. *Res Microbiol*. 2012;163(2):101-108. doi:10.1016/j.resmic.2011.11.002
114. Sato H, Feix JB. Peptide–membrane interactions and mechanisms of membrane destruction by amphipathic α-helical antimicrobial peptides. *Biochim Biophys Acta - Biomembranes*. 2006;1758(9):1245-1256. doi:10.1016/j.bbamem.2006.02.021
115. Ulmschneider JP. Charged antimicrobial peptides can translocate across membranes without forming channel-like pores. *Biophys J*. 2017;113(1):73-81. doi:10.1016/j.bpj.2017.04.056
116. Hollmann A, Martinez M, Maturana P, Semorile LC, Maffia PC. Antimicrobial peptides: interaction with model and biological membranes and synergism with chemical antibiotics. *Front Chem*. 2018;6:204. doi:10.3389/fchem.2018.00204
117. Sengupta D, Leontiadou H, Mark AE, Marrink S-J. Toroidal pores formed by antimicrobial peptides show significant disorder. *Biochim Biophys Acta - Biomembranes*. 2008;1778(10):2308-2317. doi:10.1016/j.bbamem.2008.06.007
118. Sani M-A, Separovic F. How membrane-active peptides get into lipid membranes. *Acc Chem Res*. 2016;49(6):1130-1138. doi:10.1021/acs.accounts.6b00074
119. Cardoso MH, Meneguetti BT, Costa BO, et al. Non-lytic antibacterial peptides that translocate through bacterial membranes to act on intracellular targets. *IJMS*. 2019;20(19):4877. doi:10.3390/ijms20194877
120. Benfield AH, Henriques ST. Mode-of-action of antimicrobial peptides: membrane disruption vs. intracellular mechanisms. *Front Med Technol*. 2020;2:610997. doi:10.3389/fmedt.2020.610997
121. Takeshima K, Chikushi A, Lee K-K, Yonehara S, Matsuzaki K. Translocation of analogues of the antimicrobial peptides magainin and buforin across human cell membranes. *J Biol Chem*. 2003;278(2):1310-1315. doi:10.1074/jbc.M208762200
122. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol*. 2005;3(3):238-250. doi:10.1038/nrmicro1098
123. Park CB, Kim HS, Kim SC. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem Biophys Res Commun*. 1998;244(1):253-257. doi:10.1006/bbrc.1998.8159
124. Cudic M, Otvos L Jr. Intracellular targets of antibacterial peptides. *CDT*. 2002;3(2):101-106. doi:10.2174/1389450024605445
125. Madani F, Lindberg S, Langel Ü, Futaki S, Gräslund A. Mechanisms of cellular uptake of cell-penetrating peptides. *J Biophys*. 2011;2011:1, 414729-10. doi:10.1155/2011/414729
126. Papo N, Oren Z, Pag U, Sahl H-G, Shai Y. The consequence of sequence alteration of an amphipathic α-helical antimicrobial peptide and its diastereomers. *J Biol Chem*. 2002;277(37):33913-33921. doi:10.1074/jbc.M204928200
127. Hong SY, Oh JE, Lee K-H. Effect of D-amino acid substitution on the stability, the secondary structure, and the activity of membrane-active peptide. *Biochem Pharmacol*. 1999;58(11):1775-1780. doi:10.1016/S0006-2952(99)00259-2
128. Mura M, Wang J, Zhou Y, et al. The effect of amidation on the behaviour of antimicrobial peptides. *Eur Biophys J*. 2016;45(3):195-207. doi:10.1007/s00249-015-1094-x
129. Kim J-Y, Park S-C, Yoon M-Y, Hahm K-S, Park Y. C-terminal amidation of PMAP-23: translocation to the inner membrane of gram-negative bacteria. *Amino Acids*. 2011;40(1):183-195. doi:10.1007/s00726-010-0632-1
130. Zhang F, Guo Z-L, Chen Y, Li L, Yu H-N, Wang Y-P. Effects of C-terminal amidation and heptapeptide ring on the biological activities and advanced structure of amurin-9KY, a novel antimicrobial peptide identified from the brown frog. *Rana Kunyuensis* 动物学研究. 2019;40(3):198-204. doi:10.24272/j.issn.2095-8137.2018.070
131. Ward BP, Ottaway NL, Perez-Tilve D, et al. Peptide lipidation stabilizes structure to enhance biological function. *Molec Metabol*. 2013;2(4):468-479. doi:10.1016/j.molmet.2013.08.008
132. Zhong C, Liu T, Gou S, et al. Design and synthesis of new N-terminal fatty acid modified-antimicrobial peptide analogues with potent in vitro biological activity. *Eur J Med Chem*. 2019;182:111636. doi:10.1016/j.ejmech.2019.111636
133. Domalaon R, Sanchak Y, Koskei LC, et al. Short proline-rich lipopeptide potentiates minocycline and rifampin against multidrug- and extensively drug-resistant pseudomonas aeruginosa. *Antimicrob Agents Chemother*. 2018;62(4):e02374-e02317. doi:10.1128/AAC.02374-17
134. Armas F, Di Stasi A, Mardirossian M, Romani AA, Benincasa M, Scocchi M. Effects of lipidation on a proline-rich antibacterial peptide. *IJMS*. 2021;22(15):7959. doi:10.3390/ijms22157959
135. Malina A, Shai Y. Conjugation of fatty acids with different lengths modulates the antibacterial and antifungal activity of a cationic biologically inactive peptide. *Biochem J*. 2005;390(3):695-702. doi:10.1042/BJ20050520
136. Chen T, Lyu Y, Tan M, et al. Fabrication of supramolecular antibacterial nanofibers with membrane-disruptive mechanism. *J Med Chem*. 2021;64(22):16480-16496. doi:10.1021/acs.jmedchem.1c00829
137. Lombardi L, Shi Y, Falanga A, et al. Enhancing the potency of antimicrobial peptides through molecular engineering and self-assembly. *Biomacromolecules*. 2019;20(3):1362-1374. doi:10.1021/acs.biomac.8b01740
138. Pelegri-O'Day EM, Matsumoto NM, Tamshen K, Raftery ED, Lau UY, Maynard HD. PEG analogs synthesized by ring-opening metathesis polymerization for reversible bioconjugation. *Bioconjug Chem*. 2018;29(11):3739-3745. doi:10.1021/acs.bioconjchem.8b00635
139. Dennison SR, Reddy SM, Morton LHG, Harris F, Badiani K, Phoenix DA. PEGylation enhances the antibacterial and therapeutic potential of amphibian host Defence peptides. *Biochim Biophys Acta - Biomembranes*. 2022;1864(1):183806. doi:10.1016/j.bbamem.2021.183806
140. Albada B, Metzler-Nolte N. Highly potent antibacterial organometallic peptide conjugates. *Acc Chem Res*. 2017;50(10):2510-2518. doi:10.1021/acs.accounts.7b00282
141. Eksteen JJ, Ausbacher D, Vasskog T, Rekdal Ø, Svendsen JSM. Selective intracellular delivery of thiolated cargo to tumor and neovasculature cells using histidine-rich peptides as vectors. *ACS Omega*. 2020;5(10):4937-4942. doi:10.1021/acsomega.9b00700
142. Omardien S, Drijfhout JW, Vaz FM, et al. Bactericidal activity of amphipathic cationic antimicrobial peptides involves altering the membrane fluidity when interacting with the phospholipid bilayer.

Biochim Biophys Acta - Biomembranes. 2018;1860(11):2404-2415. doi:10.1016/j.bbamem.2018.06.004
143. Kenworthy A, Preface K. *Current Topics in Membranes*. Vol. 75. Elsevier; 2015:xiii-xvii. doi:10.1016/S1063-5823(15)00033-2
144. Varshney P, Yadav V, Saini N. Lipid rafts in immune signalling: current progress and future perspective. *Immunology*. 2016;149(1):13-24. doi:10.1111/imm.12617
145. Sunshine H, Iruela-Arispe ML. Membrane lipids and cell signaling. *Curr Opin Lipidol*. 2017;28(5):408-413. doi:10.1097/MOL.0000000000000443
146. Pinigin KV, Galimzyanov TR, Akimov SA. Amphipathic peptides impede lipid domain fusion in phase-separated membranes. *Membranes*. 2021;11(11):797. doi:10.3390/membranes11110797
147. Pinigin KV, Kondrashov OV, Jiménez-Munguía I, et al. Elastic deformations mediate interaction of the raft boundary with membrane inclusions leading to their effective lateral sorting. *Sci Rep*. 2020;10(1):4087. doi:10.1038/s41598-020-61110-2
148. Rangamani P, Mandadap KK, Oster G. Protein-induced membrane curvature alters local membrane tension. *Biophys J*. 2014;107(3):751-762. doi:10.1016/j.bpj.2014.06.010
149. Franco LR, Park P, Chaimovich H, Coutinho K, Cuccovia IM, Lima FS. Simulations reveal that antimicrobial BP100 induces local membrane thinning, slows lipid dynamics and favors water penetration. *RSC Adv*. 2022;12(8):4573-4588. doi:10.1039/D1RA06267K
150. Hsu W-Y, Masuda T, Afonin S, et al. Enhancing the activity of membrane remodeling epsin-peptide by trimerization. *Bioorg Med Chem Lett*. 2020;30(12):127190. doi:10.1016/j.bmcl.2020.127190
151. Baumgart T, Hess ST, Webb WW. Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. *Nature*. 2003;425(6960):821-824. doi:10.1038/nature02013
152. García-Sáez AJ, Chiantia S, Schwille P. Effect of line tension on the lateral organization of lipid membranes. *J Biol Chem*. 2007;282(46):33537-33544. doi:10.1074/jbc.M706162200
153. Yang S-T, Kreutzberger AJB, Lee J, Kiessling V, Tamm LK. The role of cholesterol in membrane fusion. *Chem Phys Lipids*. 2016;199:136-143. doi:10.1016/j.chemphyslip.2016.05.003
154. Churchward MA, Rogasevskaia T, Höfgen J, Bau J, Coorssen JR. Cholesterol facilitates the native mechanism of Ca2+-−triggered membrane fusion. *J Cell Sci*. 2005;118(20):4833-4848. doi:10.1242/jcs.02601
155. Churchward MA, Rogasevskaia T, Brandman DM, et al. Specific lipids supply critical negative spontaneous curvature—an essential component of native Ca2+-−triggered membrane fusion. *Biophys J*. 2008;94(10):3976-3986. doi:10.1529/biophysj.107.123984
156. Tong J, Borbat PP, Freed JH, Shin Y-K. A scissors mechanism for stimulation of SNARE-mediated lipid mixing by cholesterol. *Proc Natl Acad Sci U S A*. 2009;106(13):5141-5146. doi:10.1073/pnas.0813138106
157. Kreutzberger AJB, Kiessling V, Tamm LK. High cholesterol obviates a prolonged hemifusion intermediate in fast SNARE-mediated membrane fusion. *Biophys J*. 2015;109(2):319-329. doi:10.1016/j.bpj.2015.06.022
158. Stratton BS, Warner JM, Wu Z, et al. Cholesterol increases the openness of SNARE-mediated flickering fusion pores. *Biophys J*. 2016;110(7):1538-1550. doi:10.1016/j.bpj.2016.02.019
159. Martens S, McMahon HT. Mechanisms of membrane fusion: disparate players and common principles. *Nat Rev Mol Cell Biol*. 2008;9(7):543-556. doi:10.1038/nrm2417
160. Denesvre C, Malhotra V. Membrane fusion in organelle biogenesis. *Curr Opin Cell Biol*. 1996;8(4):519-523. doi:10.1016/S0955-0674(96)80030-5
161. Jahn R, Südhof TC. Membrane fusion and exocytosis. *Annu Rev Biochem*. 1999;68(1):863-911. doi:10.1146/annurev.biochem.68.1.863
162. Apellániz B, Huarte N, Largo E, Nieva JL. The three lives of viral fusion peptides. *Chem Phys Lipids*. 2014;181:40-55. doi:10.1016/j.chemphyslip.2014.03.003
163. Moon Y, Jun Y. The effects of regulatory lipids on intracellular membrane fusion mediated by dynamin-like GTPases. *Front Cell Dev Biol*. 2020;8:518. doi:10.3389/fcell.2020.00518
164. Joardar A, Pattnaik GP, Chakraborty H. Effect of phosphatidylethanolamine and oleic acid on membrane fusion: phosphatidylethanolamine circumvents the classical stalk model. *J Phys Chem B*. 2021;125(48):13192-13202. doi:10.1021/acs.jpcb.1c08044
165. Meher G, Chakraborty H. Membrane composition modulates fusion by altering membrane properties and fusion peptide structure. *J Membr Biol*. 2019;252(4–5):261-272. doi:10.1007/s00232-019-00064-7
166. Meher G, Bhattacharjya S, Chakraborty H. Membrane cholesterol modulates oligomeric status and peptide-membrane interaction of severe acute respiratory syndrome coronavirus fusion peptide. *J Phys Chem B*. 2019;123(50):10654-10662. doi:10.1021/acs.jpcb.9b08455
167. Lozada C, Barlow TMA, Gonzalez S, Lubin-Germain N, Ballet S. Identification and characteristics of fusion peptides derived from enveloped viruses. *Front Chem*. 2021;9:689006. doi:10.3389/fchem.2021.689006
168. Millet JK, Whittaker GR. Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells. *Virology*. 2018;517:3-8. doi:10.1016/j.virol.2017.12.015
169. Cross K, Langley W, Russell R, Skehel J, Steinhauer D. Composition and functions of the influenza fusion peptide. *PPL*. 2009;16(7):766-778. doi:10.2174/092986609788681715
170. Allison SL, Schalich J, Stiasny K, Mandl CW, Heinz FX. Mutational evidence for an internal fusion peptide in flavivirus envelope protein E. *J Virol*. 2001;75(9):4268-4275. doi:10.1128/JVI.75.9.4268-4275.2001
171. Galdiero S, Vitiello M, Falanga A, Cantisani M, Incoronato N, Galdiero M. Intracellular delivery: exploiting viral membranotropic peptides. *CDM*. 2012;13(1):93-104. doi:10.2174/138920012798356961
172. Dimitrov DS. Virus entry: molecular mechanisms and biomedical applications. *Nat Rev Microbiol*. 2004;2(2):109-122. doi:10.1038/nrmicro817
173. Guillén J, de Almeida RFM, Prieto M, Villalaín J. Structural and dynamic characterization of the interaction of the putative fusion peptide of the S2 SARS-CoV virus protein with lipid membranes. *J Phys Chem B*. 2008;112(23):6997-7007. doi:10.1021/jp7118229
174. Chen YA, Scheller RH. SNARE-mediated membrane fusion. *Nat Rev Mol Cell Biol*. 2001;2(2):98-106. doi:10.1038/35052017
175. Jahn R, Scheller RH. SNAREs—engines for membrane fusion. *Nat Rev Mol Cell Biol*. 2006;7(9):631-643. doi:10.1038/nrm2002
176. Oelkers M, Witt H, Halder P, Jahn R, Janshoff A. SNARE-mediated membrane fusion trajectories derived from force-clamp experiments. *Proc Natl Acad Sci U S A*. 2016;113(46):13051-13056. doi:10.1073/pnas.1615885113
177. Robson Marsden H, Elbers NA, Bomans PHH, Sommerdijk NAJM, Kros A. A reduced SNARE model for membrane fusion. *Angew Chem Int Ed*. 2009;48(13):2330-2333. doi:10.1002/anie.200804493
178. Meyenberg K, Lygina AS, van den Bogaart G, Jahn R, Diederichsen U. SNARE derived peptide mimic inducing membrane fusion. *Chem Commun*. 2011;47(33):9405. doi:10.1039/c1cc12879e
179. Stein A, Weber G, Wahl MC, Jahn R. Helical extension of the neuronal SNARE complex into the membrane. *Nature*. 2009;460(7254):525-528. doi:10.1038/nature08156
180. Lou X, Shin Y-K. SNARE zippering. *Biosci Rep*. 2016;36(3):e00327. doi:10.1042/BSR20160004
181. Truebestein L, Leonard T. Coiled-coils: the long and short of it. *Bioessays*. 2016;38(9):903-916. doi:10.1002/bies.201600062

182. Litowski J, Hodges R. Designing heterodimeric two-stranded alpha-helical coiled-coils. *J Biol Chem*. 2002;277(40):37272-37279. doi:10.1074/jbc.M204257200
183. Versluis F, Voskuhl J, van Kolck B, et al. In situ modification of plain liposomes with lipidated coiled coil forming peptides induces membrane fusion. *J Am Chem Soc*. 2013;135(21):8057-8062. doi:10.1021/ja4031227
184. Daudey GA, Zope HR, Voskuhl J, Kros A, Boyle AL. Membrane-fusogen distance is critical for efficient coiled-coil-peptide-mediated liposome fusion. *Langmuir*. 2017;33(43):12443-12452. doi:10.1021/acs.langmuir.7b02931
185. Apostolovic B, Klok H-A. PH-sensitivity of the E3/K3 heterodimeric coiled coil. *Biomacromolecules*. 2008;9(11):3173-3180. doi:10.1021/bm800746e
186. Crone N, Minnee D, Kros A, Boyle A. Peptide-mediated liposome fusion: the effect of anchor positioning. *IJMS*. 2018;19(1):211. doi:10.3390/ijms19010211
187. Rabe M, Aisenbrey C, Pluhackova K, et al. A coiled-coil peptide shaping lipid bilayers upon fusion. *Biophys J*. 2016;111(10):2162-2175. doi:10.1016/j.bpj.2016.10.010
188. Woo SY, Lee H. All-atom simulations and free-energy calculations of coiled-coil peptides with lipid bilayers: binding strength, structural transition, and effect on lipid dynamics. *Sci Rep*. 2016;6(1):22299. doi:10.1038/srep22299
189. Koukalová A, Pokorná Š, Boyle AL, Mora NL, Kros A, Hof M. *Distinct Roles of SNARE-Mimicking Lipopeptides During Initial Steps of Membrane Fusion*:13.
190. Wagle S, Georgiev VN, Robinson T, Dimova R, Lipowsky R, Grafmüller A. Interaction of SNARE mimetic peptides with lipid bilayers: effects of secondary structure, bilayer composition and lipid anchoring. *Sci Rep*. 2019;9(1):7708. doi:10.1038/s41598-019-43418-w
191. Mora NL, Bahreman A, Valkenier H, et al. Targeted anion transporter delivery by coiled-coil driven membrane fusion. *Chem Sci*. 2016;7(3):1768-1772. doi:10.1039/C5SC04282H
192. Mora NL, Boyle AL, van Kolck BJ, et al. Controlled peptide-mediated vesicle fusion assessed by simultaneous dual-colour time-lapsed fluorescence microscopy. *Sci Rep*. 2020;10(1):3087. doi:10.1038/s41598-020-59926-z
193. Daudey GA, Shen M, Singhal A, et al. Liposome fusion with orthogonal coiled coil peptides as fusogens: the efficacy of roleplaying peptides. *Chem Sci*. 2021;12(41):13782-13792. doi:10.1039/D0SC06635D
194. Kashiwada A, Matsuda K, Mizuno T, Tanaka T. Construction of a PH-responsive artificial membrane fusion system by using designed coiled-coil polypeptides. *Chem - Eur J*. 2008;14:7343-7350.
195. Hubrich BE, Kumar P, Neitz H, et al. PNA hybrid sequences as recognition units in SNARE-protein mimicking peptides. *Angew Chem Int Ed*. 2018;57:14932-14936.
196. Ray A, Nordén B. Peptide nucleic acid (PNA): its medical and biotechnical applications and promise for the future. *FASEB j*. 2000;14(9):1041-1060. doi:10.1096/fasebj.14.9.1041
197. Gong Y, Luo Y, Bong D. Membrane activation: selective vesicle fusion via small molecule recognition. *J Am Chem Soc*. 2006;128(45):14430-14431. doi:10.1021/ja0644576
198. Sunde M, Serpell LC, Bartlam M, Fraser PE, Pepys MB, Blake CCF. Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J Mol Biol*. 1997;273:729-739.
199. Westermark P, Wernstedtt C, Wilander E, Hayden DW, O'Brient TD, Johnsont KH. Amyloid fibrils in human Insulinoma and islets of Langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells. *Proc Natl Acad Sci U S A*. 1987;5.
200. LaFerla FM, Green KN, Oddo S. *Intracellular Amyloid-β in Alzheimer's Disease*; 2007:11.
201. Spillantini MG, Schmidt ML, M.-Y. Lee V, Trojanowski JQ, Jakes R, Goedert MA. Synuclein in Lewy bodies. *Nature*. 1997;388:839-840.
202. Chiti F, Dobson CM. *Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade*. Vol. 42; 2017.
203. Eimer WA, Kumar DKV, Shanmugam NKN, et al. Alzheimer's disease-associated b-amyloid is rapidly seeded by herpesviridae to protect against brain infection. *Neuron*. 2018;99:56-63.
204. Branco MC, Sigano DM, Schneider JP. Materials from peptide assembly: towards the treatment of cancer and transmittable disease. *Curr Opin Chem Biol*. 2011;15(3):427-434. doi:10.1016/j.cbpa.2011.03.021
205. Michiels E, Roose K, Gallardo R, et al. Reverse engineering synthetic antiviral amyloids. *Nat Commun*. 2020;11(1):2832. doi:10.1038/s41467-020-16721-8
206. Zabrodskaya YA, Lebedev DV, Egorova MA, et al. The amyloidogenicity of the influenza virus PB1-derived peptide sheds light on its antiviral activity. *Biophys Chem*. 2018;234:16-23. doi:10.1016/j.bpc.2018.01.001
207. Cheetham AG, Zhang P, Lin Y, Lock LL, Cui H. Supramolecular nanostructures formed by anticancer drug assembly. *J Am Chem Soc*. 2013;135(8):2907-2910. doi:10.1021/ja3115983
208. Lin Y-A, Cheetham AG, Zhang P, et al. Multiwalled nanotubes formed by catanionic mixtures of drug amphiphiles. *ACS Nano*. 2014;8(12):12690-12700. doi:10.1021/nn505688b
209. Babych M, Bertheau-Mailhot G, Zottig X, et al. Engineering and evaluation of amyloid assemblies as a nanovaccine against the chikungunya virus. *Nanoscale*. 2018;10(41):19547-19556. doi:10.1039/C8NR05948A
210. Wen Y, Collier JH. Supramolecular peptide vaccines: tuning adaptive immunity. *Curr Opin Immunol*. 2015;35:73-79. doi:10.1016/j.coi.2015.06.007
211. Zhao G, Chandrudu S, Skwarczynski M, Toth I. The application of self-assembled nanostructures in peptide-based subunit vaccine development. *Eur Polym J*. 2017;93:670-681. doi:10.1016/j.eurpolymj.2017.02.014
212. Ni M, Zhuo S, Iliescu C, et al. Self-assembling amyloid-like peptides as exogenous second harmonic probes for bioimaging applications. *J Biophotonics*. 2019;12. doi:10.1002/jbio.201900065
213. Tsomaia N. Peptide therapeutics: targeting the undruggable space. *Eur J Med Chem*. 2015;94:459-470. doi:10.1016/j.ejmech.2015.01.014
214. Di Mauro V, Ceriotti P, Lodola F, et al. Peptide-based targeting of the L-type calcium channel corrects the loss-of-function phenotype of two novel mutations of the CACNA1 gene associated with Brugada syndrome. *Front Physiol*. 2021;11:616819. doi:10.3389/fphys.2020.616819
215. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO j*. 1999;18(7):1723-1729. doi:10.1093/emboj/18.7.1723
216. Davenport AP, Scully CCG, de Graaf C, Brown AJH, Maguire JJ. Advances in therapeutic peptides targeting G protein-coupled receptors. *Nat Rev Drug Discov*. 2020;19(6):389-413. doi:10.1038/s41573-020-0062-z
217. Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. *Front Endocrinol*. 2019;10:155. doi:10.3389/fendo.2019.00155
218. Frackenpohl J, Arvidsson PI, Schreiber JV, Seebach D. The outstanding biological stability of B- and g-peptides toward proteolytic enzymes: an in vitro investigation with fifteen peptidases. *Chem BioChem*. 2001;2:445-455. doi:10.1002/1439-7633(20010601)2:63.0.CO;2-R
219. Pahlke DM, Diederichsen U. Synthesis and characterization of β-peptide helices as transmembrane domains in lipid model membranes. *J Pept Sci*. 2016;22:636-641.

220. Rost U, Steinem C, Diederichsen U. β-Glutamine-mediated self-association of transmembrane β-peptides within lipid bilayers. *Chem Sci*. 2016;7(9):5900-5907. doi:10.1039/C6SC01147K
221. Kloos M, Sharma A, Enderlein J, Diederichsen U. Transmembrane β-peptide helices as molecular rulers at the membrane surface. *J Pept Sci*. 2021;27. doi:10.1002/psc.3355
222. Umezawa N, Gelman MA, Haigis MC, Raines RT, Gellman SH. Translocation of a β-peptide across cell membranes. *J Am Chem Soc*. 2002;124(3):368-369. doi:10.1021/ja017283v
223. Hetényi A, Szabó E, Imre N, et al. α/β-peptides as nanomolar triggers of lipid raft-mediated endocytosis through GM1 ganglioside recognition. *Pharmaceutics*. 2022;14(3):580. doi:10.3390/pharmaceutics14030580
224. Liu D, DeGrado WF. De novo design, synthesis, and characterization of antimicrobial β-peptides. *J Am Chem Soc*. 2001;123(31):7553-7559. doi:10.1021/ja0107475
225. Epand RF, Schmitt MA, Gellman SH, Epand RM. Role of membrane lipids in the mechanism of bacterial species selective toxicity by two α/β-antimicrobial peptides. *Biochim Biophys Acta - Biomembranes*. 2006;1758(9):1343-1350. doi:10.1016/j.bbamem.2006.01.018
226. Porter EA, Weisblum B, Gellman SH. Mimicry of host-defense peptides by unnatural oligomers: antimicrobial β-peptides. *J Am Chem Soc*. 2002;124(25):7324-7330. doi:10.1021/ja0260871
227. Hansen T, Alst T, Havelkova M, Strøm MB. Antimicrobial activity of small β-peptidomimetics based on the pharmacophore model of short cationic antimicrobial peptides. *J Med Chem*. 2010;53(2):595-606. doi:10.1021/jm901052r
228. Cascales L, Henriques ST, Kerr MC, et al. Identification and characterization of a new family of cell-penetrating peptides. *J Biol Chem*. 2011;286(42):36932-36943. doi:10.1074/jbc.M111.264424
229. Swedberg JE, Ghani HA, Harris JM, de Veer SJ, Craik DJ. Potent, selective, and cell-penetrating inhibitors of Kallikrein-related peptidase 4 based on the cyclic peptide MCoTI-II. *ACS Med Chem Lett*. 2018;9(12):1258-1262. doi:10.1021/acsmedchemlett.8b00422
230. Qian Z, LaRochelle JR, Jiang B, et al. Early endosomal escape of a cyclic cell-penetrating peptide allows effective cytosolic cargo delivery. *Biochemistry*. 2014;53(24):4034-4046. doi:10.1021/bi5004102
231. Mandal D, Shirazi N, Parang K. Cell-penetrating homochiral cyclic peptides as nuclear-targeting molecular transporters. *Angew Chem Int Ed*. 2011;50:9633-9637. doi:10.1002/anie.201102572
232. Rautenbach M, Vlok NM, Stander M, Hoppe HC. Inhibition of malaria parasite blood stages by tyrocidines, membrane-active cyclic peptide antibiotics from bacillus brevis. *Biochim Biophys Acta - Biomembranes*. 2007;1768(6):1488-1497. doi:10.1016/j.bbamem.2007.01.015
233. Dartois V, Sanchez-Quesada J, Cabezas E, et al. Systemic antibacterial activity of novel synthetic cyclic peptides. *Antimicrob Agents Chemother*. 2005;49(8):3302-3310. doi:10.1128/AAC.49.8.3302-3310.2005

AUTHOR BIOGRAPHIES

Ilze Lāce received her MSci in Chemistry from the University of Glasgow in 2018 under the guidance of Dr. Emily R. Draper. Currently, she is pursuing her PhD at the University of Göttingen in the group of Prof. Ulf Diederichsen and later in the group of Prof. Nadja A. Simeth. Her research explores transmembrane peptide models with photocleavable staples to Probe SNARE-induced membrane fusion.

Elena R. Cotroneo received her MSc degree from the University of Milan in 2019. She later moved to Göttingen, Germany, where she started her PhD in the group of Professor Ulf Diederichsen. She is currently a member of the group of Jr. Professor Nadja A. Simeth, working at the interface between peptide chemistry and photochemistry, with the development of light-mediated peptide systems.

Nils Hesselbarth received his master degree in chemistry from Georg-August University of Göttingen in 2020. He is currently in the third year of his PhD under the supervision of Professor Andreas Janshoff. The topic of his PhD concentrates on membrane surface anchored peptides. In particular, creating lipid rafts with exterior forces like aggregation of the peptides is of interest.

Nadja A. Simeth perused her doctorate studies with Burkhard König at the University of Regensburg as a fellow of the Studienstiftung des Deutschen Volkes and graduated in 2018 with *summa cum laude*. She afterwards joined the group of Ben L. Feringa at the University of Groningen as a postdoc supported by a Feodor-Lynen Fellowship of the Humboldt Foundation. In autumn 2021, she was appointed as assistant professor at the University of Göttingen. She is interested in the design of smart drugs, biochemical probes and labels, as well as photoresponsive supramolecular architectures and biohybrid systems.

How to cite this article: Lāce I, Cotroneo ER, Hesselbarth N, Simeth NA. Artificial peptides to induce membrane denaturation and disruption and modulate membrane composition and fusion. *J Pept Sci*. 2023;29(5):e3466. doi:10.1002/psc.3466