Modifications of Natural Peptides for Nanoparticle and Drug Design

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Abstract

Natural products serve as an important source of novel compounds for drug development. Recently, peptides have emerged as a new class of therapeutic agents due to their versatility and specificity for biological targets. Yet, their effective application often requires use of a nanoparticle delivery system. In this chapter, we review the role of natural peptides in the design and creation of nanomedicines, with a particular focus on cell-penetrating peptides, antimicrobial peptides, and peptide toxins. The use of natural peptides in conjunction with nanoparticle delivery systems holds great promise for the development of new therapeutic formulations as well as novel platforms for the delivery of various cargoes.

Keywords

Natural peptide; Nanoparticle; Drug delivery; Cell-penetrating peptide; Antimicrobial peptide; Peptide toxin

1. Introduction

Since the dawn of medicine, natural products have served as a crucial source of therapeutic compounds for drug development. From the identification of salicylic acid as the active component of willow bark extract to the discovery of penicillin produced by the mold *Penicillium rubens*, natural compounds have formed the basis of many commonly used drugs throughout history. The classical paradigm of drug development from natural products includes screening of biological extracts and identification of active components, followed by structure determination and modification. In the past 20 years, this method of developing small-molecule drugs has largely been supplanted by high-throughput screening of synthetic chemical libraries, lead compound identification, and structural optimization (Koehn & Carter, 2005). During this same time period, however, peptides have emerged as an

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important new class of drugs due to their versatility and specificity for individual targets. An enormous number of potentially therapeutic natural peptides have been identified in the course of biological research. Their clinical translation, however, has been limited by the cost of large-scale synthesis and their inefficient delivery to therapeutic sites *in vivo* (Vlieghe, Lisowski, Martinez, & Khrestchatisky, 2010). Along with continual improvements in the efficiency of peptide synthesis, nanoparticle platforms have been proposed as a strategy to facilitate delivery of peptide drugs. Conversely, natural peptides with unique biological properties have been studied as tools to enhance delivery of nanoparticles bearing other therapeutic compounds. In this chapter, we will review the use of natural peptides in the design and creation of nanomedicines, with a particular focus on short cationic or amphipathic peptides. The sequence and natural origin of several of these peptides are listed in Table 1. These compounds, which may be classified as cell-penetrating peptides, antimicrobial peptides, and peptide toxins, exhibit interesting interactions with lipid membranes which influence their use as components of nanoparticle drug delivery systems. It is important to note that there is significant overlap between members of these classes and that a particular peptide may behave as a member of more than one class. As a result, the following sections have been constructed based primarily on the applications being discussed rather than individual peptide identities. For each section, we provide a brief review of the structure and function of peptides used for these purposes followed by a detailed description of their application in nanoparticle delivery systems.

2. Role of Nanoparticles in Peptide Drug Delivery

Despite a great deal of enthusiasm, there remain several key pharmacological limitations to the development of peptide-based pharmaceutical agents (Craik, Fairlie, Liras, & Price, 2013). Short peptides (< 50 amino acids) have poor pharmacokinetic profiles due to their degradation by serum proteases and rapid clearance by renal filtration. Additionally, most peptide drugs are limited to extracellular targets due to their inability to cross the plasma membrane and, like all drugs, may have off-target effects when administered systemically. Nanoparticle delivery platforms hold great promise as tools to overcome these limitations and enhance the efficacy and utility of peptide drugs. The term "nanoparticle" refers to an extremely broad range of constructs between 1 and 500 nm in diameter which possess unique physical, chemical, and biological properties as a result of their size (Caruthers, Wickline, & Lanza, 2007). Although most types of nanoparticles have been applied in some way for drug delivery, lipid and polymer nanoparticles are by far the most extensively studied and commonly used for this purpose. The rest of this chapter will focus on the use of these nanoparticles as a platform for the delivery of natural peptides and their derivatives.

Lipid nanoparticles as a class include liposomes and perfluorocarbon nanoparticles, as well as a number of other lipid-based constructs. Liposomes are among the most well-studied nanostructures for drug delivery and include several formulations currently approved for clinical use (Allen & Cullis, 2013). As shown in Figure 1, liposomes are vesicles consisting of a phospholipid bilayer surrounding an aqueous core (Figure 1A). Based on their hydrophobicity, small-molecule compounds may either be encapsulated within the aqueous region (e.g., liposomal doxorubicin, Doxil®) or incorporated into the lipid bilayer (e.g., liposomal amphotericin B, AmBisome®). Perfluorocarbon nanoparticles, in contrast, consist

of a hydrophobic perfluorocarbon core stabilized by a phospholipid monolayer shell (Figure 1B). These nanoparticles have been extensively used for targeted drug delivery and imaging via incorporation of targeting ligands into the phospholipid monolayer (Tran et al., 2007). For both liposomes and perfluorocarbon nanoparticles, peptides may be covalently attached to the phospholipid head groups or non-covalently associated with the lipid membrane. Due to their relatively large size, liposomes and perfluorocarbon nanoparticles alter the pharmacokinetic profile of attached peptides, preventing renal filtration and leading to an overall increase in the circulating peptide half-life. For instance, covalent attachment of the thrombin inhibitor D-phenylalanyl-L-prolyl-L-arginyl-chloromethylketone (PPACK) to the surface of liposomes (Palekar et al., 2013) or perfluorocarbon nanoparticles (Myerson, He, Lanza, Tollefsen, & Wickline, 2011) substantially increased its duration of antithrombotic activity relative to free PPACK. Additionally, insertion into the lipid monolayer of perfluorocarbon nanoparticles enhanced circulation time of melittin, a cytolytic peptide toxin derived from bee venom (Soman et al., 2009). Loading of melittin onto perfluorocarbon nanoparticles also protected melittin from cleavage by serum proteases and significantly reduced its hemolytic activity and toxicity when injected systemically. Interestingly, attempts to use liposomes as a melittin delivery vehicle resulted in membrane pore formation and complete disruption of liposome structure (Soman, Lanza, Heuser, Schlesinger, & Wickline, 2008). These findings illustrate the importance of selecting an appropriate nanoparticle carrier to enhance the therapeutic potential of peptide agents.

Polymer nanoparticles are extremely versatile drug carriers which may be generated using a wide variety of natural or synthetic polymers. Biodegradable synthetic polymers, such as poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone), often aggregate to form nanoparticles via hydrophobic interactions (Figure 1C). Additionally, several methods have been developed to synthesize nanoparticles composed of natural hydrophilic polymers, such as chitosan and gelatin (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). One important characteristic of polymer nanoparticles is the ability to directly modify the polymer chain as needed to achieve desired functionality. For instance, PLGA nanoparticles containing polymer chains grafted with poly(ethylene glycol) and a cyclic RGD targeting peptide demonstrated enhanced delivery of paclitaxel and doxorubicin to a variety of *in vitro* and *in vivo* tumor models (Danhier et al., 2012). Peptide cargoes may be either covalently linked to the polymer backbone in this way or non-covalently associated with the polymer matrix via electrostatic and hydrophobic interactions. Interestingly, amphipathic peptides may self-assemble into nanoparticles, thereby facilitating their own delivery and that of any complexed cargo. Cell-penetrating peptides in particular have been used in this fashion to enhance translocation of nucleotides and other peptides across the cell membrane (Heitz, Morris, & Divita, 2009).

3. Cell-penetrating peptides

In order for macromolecules and nanoparticles to exert therapeutic effects intracellularly, they must first cross the plasma membrane. Over the past 25 years, a large amount of work has focused on the development of peptide agents capable of facilitating this process, leading to enhanced cellular uptake of cargoes ranging from individual protein molecules (Schwarze, Ho, Vocero-Akbani, & Dowdy, 1999) to liposomes with diameters of 200 nm

(Torchilin, Rammohan, Weissig, & Levchenko, 2001). Many of these cell-penetrating peptides (CPPs) are originally derived from natural proteins which translocate across biological membranes. The first reports of this phenomenon came from studies of the HIV-1 *trans*-activator of transcription (Tat) protein, which penetrates membranes and accumulates in the nucleus by an energy-independent mechanism (Frankel & Pabo, 1988; Green & Loewenstein, 1988). Further studies identified the minimal peptide sequence required for this uptake (Vivès, Brodin, & Lebleu, 1997) and led to the discovery of other CPPs such as penetratin, derived from the homeodomain of the *Drosophila* Antennapedia protein (Derossi, Joliot, Chassaing, & Prochiantz, 1994). Since then, a wide variety of CPPs have been identified from sources such as viral proteins (Elliott & O'Hare, 1997), animal venoms (Hou, Pan, Lanza, & Wickline, 2013) and synthetic peptide libraries (Gao, Simon, Hue, Morrison, & Banta, 2011). These diverse peptides are typically 5–30 amino acids in length and possess a variety of structural and chemical properties which impact their use as macromolecule and nanoparticle delivery agents. In this section, we will review structural characteristics of the most commonly used CPPs and examine their use in nanoparticle-based drug delivery systems. For a more comprehensive listing of CPPs, the reader is referred to the review by Milletti (Milletti, 2012).

3.1. Structure and Properties

The number and variety of CPPs reported in the literature make precise classification difficult. CPPs may be broadly divided into groups based on multiple considerations, including origin, sequence, and proposed mechanism of internalization. In particular, classification by sequence provides a logical framework for understanding the structure and function of CPPs, as well as their role in the design of nanostructures. In this system, CPPs are classified as either cationic, amphipathic, or hydrophobic.

Cationic CPPs—Cationic CPPs contain positively charged residues which are essential for uptake and often adopt random coil configurations both in solution and when associated with lipid membranes (Eiríksdóttir, Konate, Langel, Divita, & Deshayes, 2010). While the Tat peptide is the most commonly used member of this class, many other cationic CPPs have been identified from sources such as heparan binding proteins (de Coupade et al., 2005), bacteriophages (Futaki et al., 2001), and transcription factors (Balayssac et al., 2006). Internalization of cationic CPPs relies heavily on the presence of arginine residues, which are thought to interact strongly with anionic cell surface proteoglycans. In fact, polyarginine sequences themselves can serve as CPPs, with several studies identifying octaarginine as the minimal sequence for cellular uptake (El-Sayed, Futaki, & Harashima, 2009; Tünnemann et al., 2008). Following interaction with the cell surface, the mechanisms of CPP uptake become controversial (Heitz et al., 2009; Richard et al., 2003). Although one or more endocytotic mechanisms are likely involved, the contribution of each mechanism may depend on the particular CPP being studied and the cargo to which it is bound (Duchardt, Fotin-Mleczek, Schwarz, Fischer, & Brock, 2007).

Amphipathic CPPs—Amphipathic CPPs contain both hydrophilic and hydrophobic domains which are necessary for cellular internalization. In primary amphipathic CPPs, these segments are separated based on their position along the peptide chain. This class

includes several natural peptides such as pVEC, which is derived from VE-cadherin (Elmquist, Lindgren, Bartfai, & Langel, 2001). Primary amphipathic CPPs may be designed as fusion constructs containing the targeting domain of one protein and the membrane-interacting domain of another. For instance, both MPG and Pep-1 contain the cationic Simian Virus 40 (SV40) nuclear localization sequence. However, MPG contains a hydrophobic domain derived from the fusion sequence of HIV-1 glycoprotein 41, while that of Pep-1 is derived from the dimerization motif of HIV-1 reverse transcriptase (May C. Morris, Depollier, Mery, Heitz, & Divita, 2001; M. C. Morris, Vidal, Chaloin, Heitz, & Divita, 1997). These different hydrophobic domains allow MPG to carry nucleic acid cargoes while Pep-1 preferentially complexes with other peptides and proteins (Deshayes, Morris, Divita, & Heitz, 2005).

Secondary amphipathic CPPs are another large class of peptides in which the separation between hydrophilic and hydrophobic domains occurs due to the formation of secondary structures, such as α-helices or β-sheets. Many of the most commonly used CPPs are members of this class. These include penetratin (Derossi et al., 1994), which is derived from the Antennapedia homeodomain, and transportan (Pooga, Hällbrink, Zorko, Uuml, & Langel, 1998), which is derived from fusion of the neuropeptide galanin with the wasp venom peptide mastoparan. Many antimicrobial peptides and animal toxins also resemble amphipathic CPPs in structure. In order for these peptides to be successfully used for drug delivery, however, they must first be modified to minimize their cytotoxicity. Although most amphipathic CPPs are cationic, there are a number of neutral and anionic peptides which retain their cell-penetrating ability (W. Li, Nicol, & Szoka Jr., 2004; Taylor et al., 2009). These findings indicate that cellular internalization is a consequence of these peptides' amphipathic nature, not solely their charge.

Hydrophobic CPPs—Hydrophobic CPPs possess a low net charge and are composed of predominantly nonpolar amino acids. Several hydrophobic CPPs are derived from natural proteins, including α1-antitrypsin (Rhee & Davis, 2006) and fibroblast growth factor 12 (Nakayama et al., 2011). Nevertheless, this class includes the fewest CPPs discovered to date and most new hydrophobic CPPs are generated using synthetic peptide libraries (Marks, Placone, Hristova, & Wimley, 2011). This class also includes a number of chemically modified peptides, such as stapled peptides (Chu et al., 2014), prenylated peptides (Ochocki, Mullen, Wattenberg, & Distefano, 2011), and pepducins (Covic, Gresser, Talavera, Swift, & Kuliopulos, 2002). The use of these synthetic modifications to enhance peptide uptake raises exciting prospects for the development of cell-penetrating agents based on a wide variety of natural products.

3.2. Applications in Nanoparticle Delivery

Although nanoparticles possess several distinct advantages as drug delivery vehicles, their size generally prevents them from crossing the plasma membrane, thereby precluding their use for intracellular drug delivery. One promising approach to overcome this limitation involves incorporating CPPs directly into the nanoparticle structure. Depending on the type of nanoparticle, this conjugation may be either covalent or non-covalent.

Multiple studies have demonstrated that covalent addition of cationic or amphipathic CPPs to the surface of nanostructures is sufficient to enhance their cellular uptake. This technique has been successfully applied to a wide range of nanoparticles, including liposomes (Tseng, Liu, & Hong, 2002), iron oxide nanoparticles (M. Zhao, Kircher, Josephson, & Weissleder, 2002), gold nanoparticles (de la Fuente & Berry, 2005), and quantum dots (Ruan, Agrawal, Marcus, & Nie, 2007). Advantages of covalent conjugation include its simplicity, stability and applicability to a variety of well-defined nanoparticles. Nevertheless, continued interaction between the CPP and cargo following translocation into the cell may diminish the cargo's biological activity (Juliano, Alam, Dixit, & Kang, 2008). In these cases, the use of cleavable linkers or non-covalent association of the CPP may be more appropriate.

The non-covalent approach for CPP incorporation relies on electrostatic and hydrophobic interactions between the CPP and cargo to maintain nanoparticle integrity. This technique was originally developed using the fusion peptide of influenza hemagglutinin, which condenses with DNA to form nanoscale complexes capable of escaping the endosome (Wagner, Plank, Zatloukal, Cotten, & Birnstiel, 1992). Recently, this approach has been extended to a number of other CPPs and has been successfully used for the delivery of nucleotide (Hou, Pan, Lanza, et al., 2013; Nakase et al., 2012), peptide (Grdisa, 2011) and small-molecule therapeutics (L. Liu et al., 2008). The wide variety of CPPs, nanoparticles, and conjugation methods available provides great freedom in the design of drug delivery systems. For the purposes of this chapter, we have classified studies based on the specific CPP used in each delivery system.

Tat Peptide—One of the most commonly used CPPs in the field of drug delivery is the Tat peptide, an 11-mer identified as the minimal sequence necessary for membrane translocation of the HIV-1 *trans*-activator of transcription (Tat) protein (Vivès et al., 1997). The first application of this peptide to enhance nanoparticle uptake involved its covalent attachment to the surface of superparamagnetic iron oxide nanoparticles (SPIO NPs) (Josephson, Tung, Moore, & Weissleder, 1999). SPIO NPs bearing the Tat peptide were internalized over 100-fold more efficiently than control nanoparticles, allowing labeled cells to be detected by magnetic resonance imaging and retained on magnetic separation columns. A similar technique was used by Torchilin et al. to enhance uptake of 200-nm liposomes by a variety of cell lines (Torchilin et al., 2001). This and other studies (Marty, Meylan, Schott, Ballmer-Hofer, & Schwendener, 2004) demonstrated that liposome uptake was dependent on the number of peptides attached to the liposome as well as their ability to interact with the plasma membrane and cell surface proteoglycans. Covalent functionalization with Tat peptide has subsequently been applied to a variety of lipid-based drug delivery systems. Liposomes are well-established as carriers to increase the efficacy and reduce off-target toxicity of chemotherapeutic agents (A. Z. Wang, Langer, & Farokhzad, 2012). Tseng et al. demonstrated that addition of Tat peptide to the liposomal surface increased uptake of doxorubicin by 12-fold in a number of cancer cell lines (Tseng et al., 2002). Interestingly, however, they also found that this enhanced uptake was not accompanied by increased cytotoxicity or inhibition of tumor growth in a subcutaneous xenograft model. Other studies have reported somewhat more positive results, with Qin et al. claiming that Tat peptide-conjugated liposomes enhanced cytotoxicity and moderately improved survival in a mouse

model of brain glioma (Qin et al., 2011). In either case, limited penetration into the tumor and slow release of encapsulated drug have been identified as key obstacles to liposomal drug delivery (Manzoor et al., 2012) and may persist even with the use of CPPs. Nanostructured lipid carriers functionalized with Tat peptide have also been used in transdermal applications to deliver the non-steroidal anti-inflammatory drug celecoxib (Desai, Patlolla, & Singh, 2010). These agents significantly increased celecoxib accumulation in all skin layers compared to control carriers lacking the Tat peptide.

The first application of CPPs for nanoparticle-based gene delivery involved the use of lipoplexes formed by electrostatic interaction of Tat peptide-modified cationic liposomes with DNA (Torchilin et al., 2003). These nanoparticles were used to transfect fibroblasts and cardiomyocytes *in vitro*, as well as Lewis lung carcinoma cells growing subcutaneously *in vivo*. Similar lipoplexes demonstrated successful gene delivery to brain tumors (Gupta, Levchenko, & Torchilin, 2007) and ischemic myocardium (Ko, Hartner, Kale, & Torchilin, 2008) in animal models. Recently, polymer nanostructures have gained popularity as nucleotide delivery vehicles. These carriers often contain Tat peptide covalently linked to a cationic polymer which forms nanoscale complexes with nucleotides. Kleeman et al. demonstrated this concept using a complex of DNA with a Tat-peptide conjugated form of polyethyleneimine (Kleemann et al., 2005). The resulting polyplexes successfully transfected lung carcinoma cells *in vitro*, as well as bronchial and alveolar tissue following intratracheal instillation. A similar nanoparticle was later used to transfect neurotypic cells with possible implications for treatment of neurodegenerative diseases (Suk et al., 2006). Tat peptide-functionalized chitosan nanoparticles have also been used for this application and have successfully delivered siRNA to reduce *in vitro* production of ataxin, a protein overexpressed in spinocerebellar ataxia (Malhotra, Tomaro-Duchesneau, & Prakash, 2013). While Tat peptide-conjugated polymer carriers are often used for gene delivery, they have also been applied as alternatives to liposomes for the delivery of paclitaxel (Sawant & Torchilin, 2009; P. Zhao et al., 2010) and doxorubicin (Lee et al., 2011) to tumors, as well as the delivery of antibiotics such as ciprofloxacin (L. Liu et al., 2008) across the blood-brain barrier.

One major drawback to the use of CPPs for drug delivery is their lack of specificity for a particular tissue or cell type. An early mouse study which involved intraperitoneal injection of a Tat peptide-β-galactosidase fusion protein found that this construct accumulated in all tissues analyzed, including the brain (Schwarze et al., 1999). While this property may be desirable for some applications, such as systemic gene delivery, it could result in off-target effects and increased dose requirement for many drug delivery systems. Thus, recent efforts using Tat peptide have focused on developing nanocarriers in which the internalizing effects of the CPP are activated only under specific conditions. Kale et al. developed one such system in which liposomes were functionalized with both Tat peptide and pH-sensitive poly(ethylene glycol) (PEG) chains (Kale & Torchilin, 2007a, 2007b). The PEG coating sterically hindered membrane interaction of Tat peptide, thereby preventing liposome internalization until the PEG chains were hydrolyzed in the acidic tumor microenvironment. A similar concept has been applied to liposomes bearing a monoclonal antibody to enhance interaction with tumor cells (Koren, Apte, Jani, & Torchilin, 2012). These multifunctional

liposomes enhanced delivery of doxorubicin to ovarian cancer both *in vitro* and *in vivo* (Apte, Koren, Koshkaryev, & Torchilin, 2014). Drug delivery systems activated by tumor-associated proteases have also been developed. Gullotti et al. reported a Tat peptide-functionalized polymer nanoparticle bearing PEG chains anchored by a matrix metalloproteinase-2 (MMP-2) cleavable peptide sequence (Gullotti, Park, & Yeo, 2013). Treatment with MMP-2 led to increased nanoparticle uptake and delivery of paclitaxel in an ovarian cancer cell line. Similarly, Liu et al. generated a Tat peptide-modified liposome activated by legumain, a protease overexpressed in a variety of tumor tissues (Z. Liu et al., 2014). In this study, however, the cleavable group was covalently linked to Tat peptide, reducing its cell-penetrating activity prior to cleavage by legumain. These strategies for CPP activation may be used in conjunction with other techniques to locally enhance nanoparticle uptake. The application of ultrasound energy, for instance, improved the ability of Tat peptide-modified bubble liposomes to escape the endosome, leading to a 30-fold increase in gene delivery (Omata et al., 2011).

Penetratin—Penetratin, one of the first CPPs to be discovered, is a 16-mer peptide derived from the third helix of the *Drosophila* Antennapedia homeodomain (Derossi et al., 1994). It is commonly used as an alternative or complement to Tat peptide for enhancing cellular uptake of nanoparticles. Like Tat peptide, penetratin is often covalently linked to the nanoparticle surface. One of the most common applications for penetratin-modified nanoparticles is to facilitate drug delivery across the blood-brain barrier. Xia et al. found that polymer nanoparticles functionalized with penetratin demonstrated increased cellular uptake *in vitro* and improved pharmacokinetics *in vivo* (Xia et al., 2012). These nanoparticles accumulated to a greater extent in the brain and to a lesser extent in other tissues than unmodified nanoparticles. Similarly, addition of penetratin to the surface of liposomes improved liposomal accumulation and delivery of encapsulated doxorubicin to brain tumors (Sharma, Modgil, Zhong, Sun, & Singh, 2014). Penetratin-functionalized polymer nanoparticles have also been used to deliver peptide nucleic acids, a new class of therapeutics, to malignant lymphocytes in a mouse model of lymphoma (Babar et al., 2012). Interestingly, this study found that pre-B cells preferentially internalized nanoparticles coated with penetratin when compared to other CPPs, such as Tat peptide and polyarginine.

MPG/Pep-1—Covalent linkage of Tat peptide and penetratin has been shown to inhibit both cellular uptake and biological activity of certain types of cargo, particularly nucleotides (Presente & F. Dowdy, 2013). To avoid this limitation, several CPPs have been designed which form non-covalent nanoscale complexes with cargo via electrostatic and hydrophobic interactions. These complexes enhance cellular internalization while protecting cargo from degradation by serum nucleases and proteases. MPG is an amphipathic peptide generated by using a flexible peptide linker to join the hydrophobic fusion sequence of HIV-1 glycoprotein 41 with the cationic nuclear localization sequence of Simian Virus 40 (SV40) large T antigen (M. C. Morris et al., 1997). This peptide forms 200-nm complexes with nucleic acids and was initially used for the delivery of oligonucleotides and plasmid DNA into cultured cells (May C. Morris, Chaloin, Méry, Heitz, & Divita, 1999). More recently, the sequence of this construct was optimized for siRNA delivery by introducing a point mutation to the nuclear localization sequence, thereby facilitating nanoparticle accumulation

and siRNA release in the cytoplasm (Simeoni, Morris, Heitz, & Divita, 2003). Although this and other MPG derivatives have been successfully used for gene delivery to a variety of cell lines *in vitro*, it appears that endosomal entrapment remains an important limitation to transfection efficiency (Veldhoen, Laufer, Trampe, & Restle, 2006). For *in vivo* applications, biodistribution presents yet another potential obstacle to effective gene delivery. To address this issue, Crombez et al. functionalized an MPG derivative with cholesterol and demonstrated that this modification improved tissue distribution following systemic administration in mice (Crombez et al., 2009). The use of this system to deliver cyclin B1 siRNA reduced tumor growth and increased survival following intravenous injection in a mouse xenograft cancer model.

Given the success of MPG for enhancing nucleotide delivery, Morris et al. developed a similar peptide which facilitates uptake of proteins via formation of non-covalent complexes (May C. Morris et al., 2001). This peptide, known as Pep-1, contains the same hydrophilic domain and peptide linker as MPG, but a different hydrophobic domain derived from a tryptophan-rich cluster found in HIV-1 reverse transcriptase. A study of Pep-1 complexation with the cell cycle inhibitor protein p27^{Kip1} found an optimal Pep-1:p27 molar mixing ratio of 20:1, which resulted in discrete nanoparticles with diameters of approximately 100 nm (Muñoz-Morris, Heitz, Divita, & Morris, 2007). Addition of these complexes to cultured fibroblasts led to cell cycle arrest in 70% of cells analyzed. Pep-1 has been used to deliver proteins to a wide variety of cell lines (Gros et al., 2006), including primary neurons (Gallo, 2003), hepatocytes (Bardag-Gorce et al., 2003), and macrophages (Garnon et al., 2005). This peptide has also been commercialized for research purposes under the trade name Chariot™ (Active Motif, Carlsbad, CA). *In vivo* applications of Pep-1 have been promising, with successful protein delivery demonstrated following intratracheal instillation in a mouse model of emphysema (Aoshiba, Yokohori, & Nagai, 2003). Recently, however, Pep-1 has primarily been used as a covalent attachment to facilitate penetration of nanoparticles into tumors (Kang, Park, Kang, Park, & Choi, 2013; B. Wang et al., 2014) and generate fusion proteins with enhanced tissue accumulation (Ahn et al., 2014; He et al., 2013). These applications resemble those of other CPPs and demonstrate the versatility of Pep-1 as a tool for improving drug delivery.

Antimicrobial Peptides—Like CPPs, antimicrobial peptides (AMPs) often possess cationic or amphipathic segments which enhance their interaction with lipid membranes. Indeed, some authors have proposed that both CPPs and AMPs belong to a class of "membrane-active peptides", with their primary distinguishing feature being the extent of membrane translocation vs. permeabilization induced at a particular peptide concentration (Henriques, Melo, & Castanho, 2006). Several AMPs have been reported to exhibit cell-penetrating properties (Splith & Neundorf, 2011). However, their practical application as drug delivery vehicles remains limited by their toxicity to mammalian cells. Judicious peptide sequence modifications may substantially reduce cytotoxicity while maintaining cell-penetrating activity. For instance, Pan et al. reported that N-terminal truncation of melittin, an AMP derived from bee venom, reduced cytotoxicity by two orders of magnitude without significantly affecting the ability of melittin to insert into lipid membranes (Pan et al., 2010). Addition of arginine and histidine residues to the C-terminus of this peptide gave

rise to p5RHH, a transfection agent capable of forming nanoscale complexes with siRNA (Hou, Pan, Lanza, et al., 2013). As shown in Figure 2, these complexes are stable in serum and have been successfully used to transfect macrophages, B16 melanoma cells, and human umbilical vein endothelial cells *in vitro*. Mechanistic studies indicated that endosomal acidification led to disassembly of these complexes and lysis of the endosomal membrane, thereby facilitating siRNA release into the cytoplasm (Hou, Pan, Ratner, Schlesinger, & Wickline, 2013). These complexes accumulated at inflammatory sites following intravenous injection in a mouse model of rheumatoid arthritis. Incorporation of siRNA targeting the p65 subunit of NF-κB led to dramatic improvements in joint inflammation without eliciting a humoral immune response or affecting p65 expression in other tissues (Zhou et al., 2014).

4. Antimicrobial peptides

With the rise of antibiotic resistance and the emergence of multidrug-resistant organisms (Andersson & Hughes, 2011; Bush et al., 2011), there is a pressing need to develop new antimicrobial agents which avoid conventional mechanisms of resistance. One promising set of compounds which could form the basis for a new class of therapeutics are antimicrobial peptides (AMPs). These peptides are produced by a wide variety of organisms and generally exert their antimicrobial effect by disrupting biological membranes, leading to depolarization, lysis, and cellular death. Like cell-penetrating peptides, AMPs are often short (10–50 amino acids), cationic (overall charge +2 to +9), and possess a number of hydrophobic residues to facilitate their interaction with lipid membranes (Hancock & Sahl, 2006). This class of compounds is also extremely diverse, with over 1,200 peptides divided into five structural classes (G. Wang, Li, & Wang, 2009) and a variety of proposed mechanisms of action (Nguyen, Haney, & Vogel, 2011). In contrast to conventional antibiotics, AMPs do not target a specific molecule or biochemical process, giving them broad-spectrum activity and making development of resistance difficult (Zasloff, 2002). Nevertheless, there are some important limitations preventing clinical translation of these compounds. While many natural AMPs demonstrate some degree of preference for bacterial membranes, toxicity to eukaryotic cells remains a key concern (Marr et al., 2006). Additionally, like many short peptides, free AMPs are rapidly cleared by the kidney and possess a poor pharmacokinetic profile (Bradshaw, 2003). Recent efforts to address these concerns include sequence modifications to enhance antimicrobial activity while minimizing toxicity to eukaryotic cells, as well as development of nanoparticle-based strategies to improve AMP delivery while further reducing off-target treatment effects.

4.1. Sequence Modifications

As with cell-penetrating peptides, the ability of an AMP to disrupt biological membranes depends primarily on its general structure and physicochemical properties rather than its specific amino acid composition. Thus, sequence modifications intended to change peptide activity often alter key peptide characteristics such as charge and hydrophobicity. Addition of cationic residues to either the N- or C-terminus of magainin II, an AMP derived from the African clawed frog, substantially improved its antibacterial activity without significantly altering its hemolytic activity (Bessalle, Haas, Goria, Shalit, & Fridkin, 1992). This effect was attributed to enhanced electrostatic interaction between the magainin derivatives and

bacterial membranes, which contain high concentrations of anionic phospholipids. A similar trend was observed using cationic derivatives of the AMP V_{681}, which is derived from a fusion construct of cecropin A, an AMP isolated from the *Hyalophora cecropia* moth, and melittin, an AMP found in bee venom (Jiang et al., 2008). However, this study also identified a charge threshold above which hemolytic activity of the V_{681} derivatives sharply increased.

The effects of hydrophobicity on antibacterial and hemolytic activity are similarly difficult to predict. Replacing two leucine residues with more hydrophobic hexafluoroleucine residues enhanced antibacterial activity without altering hemolytic activity in buforin II, an AMP derived from stomach tissue of the Asian toad (Meng & Kumar, 2007). However, similar alterations of the magainin sequence increased hemolytic activity without substantially affecting antibacterial activity. Many other sequence modifications have been applied to a wide variety of AMPs in order to enhance their selectivity for bacterial membranes or gain information regarding their mechanism of action. For a more comprehensive assessment of these efforts, the reader is referred to the review by Findlay et al. (Findlay, Zhanel, & Schweizer, 2010).

Modification of an AMP sequence may also be performed to introduce properties which are beneficial for a particular application. For instance, Pan et al. recently developed a peptide linker to non-covalently attach cargo to the surface of lipid nanoparticles (Pan et al., 2010). This linker is generated by deleting seven amino acids from the N-terminus of the AMP melittin. The resulting peptide is substantially less cytotoxic and hemolytic than free melittin but retains the ability of melittin to stably bind and insert into lipid membranes. A fusion construct of this linker with the Nemo Binding Domain peptide was used to generate perfluorocarbon nanoparticles which potently inhibited NF-κB signaling in leukemia/ lymphoma cells (Pan et al., 2011). Additionally, this linker was used to create perfluorocarbon nanoparticles targeted to the inflammatory marker VCAM-1 (Pan et al., 2013). These nanoparticles accumulated in atherosclerotic plaques and breast tumors to a much greater extent than untargeted nanoparticles, making them promising vehicles for drug delivery to these sites.

While truncation of an AMP sequence permanently reduces its cytotoxicity, AMP activity may be reversibly inhibited by addition of a removable anionic blocking sequence. Desgranges et al. demonstrated this concept using a cecropin-magainin hybrid peptide containing an N-terminal polyglutamate sequence joined via a trialanine linker (Desgranges et al., 2011). This linker sequence was designed to be cleaved by neutrophil elastase, leading to activation of cytolytic activity only in the presence of inflammation. While this compound exhibited potent activity against *Pseudomonas aeruginosa* following incubation with recombinant neutrophil elastase, activation by bronchoalveolar lavage specimens was much less pronounced despite the presence of similar elastase concentrations (Forde, Humphreys, Greene, Fitzgerald-Hughes, & Devocelle, 2014). This effect resulted from inhibition of cytolytic activity by anionic components of the bronchoalveolar lavage sample, demonstrating the complexity of AMP development for clinical applications.

4.2. Nanoparticle Delivery Strategies

In addition to sequence modifications, nanoparticle delivery vehicles may be used to minimize off-target effects and improve AMP release kinetics. For instance, incorporation of melittin into the lipid monolayer of perfluorocarbon nanoparticles has been shown to reduce toxicity to sperm and vaginal epithelium (Jallouk et al., 2014) while retaining antiviral activity against HIV-1 (Hood, Jallouk, Campbell, Ratner, & Wickline, 2013). This preferential inactivation of HIV-1 virions was attributed to their relatively small surface area, high internal pressure, and inability to repair membrane defects. These findings demonstrated the potential utility of melittin-loaded nanoparticles as a topical vaginal virucide. Similarly, LL-37, an AMP derived from human cathelicidin, retained activity against *Escherichia coli* following incorporation into polymer nanoparticles (Chereddy et al., 2014). Intradermal administration of LL-37-loaded nanoparticles led to sustained LL-37 release and improved wound healing in a mouse injury model.

Like cell-penetrating peptides, amphipathic AMPs may self-assemble to form nanoparticles via electrostatic and hydrophobic interactions (Hamley, 2011; X. Zhao et al., 2010). This self-assembly process, in turn, may be influenced through covalent addition of cationic or hydrophobic groups to the peptide sequence. Liu et al. generated one such peptide, known as CG_3R_6TAT, which consists of cholesterol joined via a triglycine linker to a hexaarginine-modified version of Tat peptide (L. Liu et al., 2009). As shown in Figure 3, this construct self-assembles into 150-nm nanoparticles with core-shell morphology. The presence of a cell-penetrating peptide modified with additional cationic and hydrophobic groups enhances the ability of these nanoparticles to disrupt and translocate across biological membranes. These nanoparticles exhibited antimicrobial activity against a wide range of bacteria, yeast, and other fungi *in vitro*, and demonstrated an antibacterial effect in a rabbit model of *Staphylococcus aureus*-induced meningitis. In a more detailed study using a rabbit model of *Cryptococcus neoformans*-induced meningitis, these nanoparticles were shown to penetrate the blood-brain barrier and appeared to have an antifungal effect similar to amphotericin B with fewer off-target effects on liver and kidney function (Huaying Wang et al., 2010).

5. Peptide toxins

In addition to their primary role in defense from predators and capture of prey, animal venoms represent a potent reservoir of potentially bioactive molecules with broad therapeutic applications (Bailey & Wilce, 2001). Venom is composed of many different bioactive peptides which contribute to the pharmacologic response after delivery to the recipient (Lewis & Garcia, 2003). Due to their high potency and specificity, venom peptides remain an attractive tool for probing the structure and function of their various targets, including voltage-gated ion channels, nicotinic acetylcholine receptors, N-methyl-D-aspartate (NMDA) receptors, and acid-sensing ion channels (ASICs) (Dutertre & Lewis, 2010). Additionally, venom peptides have been used as structural templates for the development of drugs targeting these receptors, many of which have been successfully translated to the clinic. In this section, we will discuss venom peptides derived from several different organisms, as well as their potential therapeutic applications.

5.1. Mechanisms of Action

The mechanism of action of individual peptide toxins varies greatly based on the organism from which they are derived and their intended biological effect. Nevertheless, many peptides derived from animal venoms are toxic to bacteria, fungi and animal cells by virtue of their ability to disrupt biological membranes. The exact mechanism of membrane disruption is heavily debated and can vary based on the peptide being studied. However, most proposed mechanisms are based on several commonly accepted models (Shai, 1999). In the "carpet" model, peptides in an α-helical conformation coat the membrane and alter its curvature in a detergent-like fashion. Increased peptide deposition eventually leads to collapse of the membrane and leakage of its contents. In the "barrel stave" model, peptides bind electrostatically to membrane phospholipids and oligomerize, resulting in the formation of transmembrane pores and osmotic lysis. In the "toroidal pore" model, insertion of peptides into the membrane results in conformational strain leading to increased distance between phospholipid head groups, cavitation, pore formation, and osmotic lysis. This disruption of biological membranes forms the basis for therapeutic application of a number of peptide toxins.

5.2. Therapeutic Applications

Amphibian Toxins—Toxins from amphibians, particularly frogs and toads, are typically delivered as a secretion from the skin, where they play a major role in defending the host from invasion by potentially lethal bacteria and fungi. Studies of skin secretions from amphibians have isolated several potential therapeutic agents with antimicrobial properties. Like other antimicrobial peptides, these toxins have been studied for their ability to disrupt bacterial membranes and induce cell death. The first of these peptides to be isolated was magainin, derived from the African clawed frog *Xenopus laevis*. Additional work has resulted in a library of more than 300 cytolytic peptides found in skin secretions, ranging from 10–48 amino acids in length and generally cationic due to the presence of several lysine residues (Conlon, Kolodziejek, & Nowotny, 2004). Toxins commonly studied for their antimicrobial activity include dermaseptin, dermatoxin, distinctin, phylloseptin, phylloxin, and plasticins, among others (Calderon, Silva, Ciancaglini, & Stábeli, 2011). Despite their efficacy as antimicrobial agents, clinical translation of many amphibian toxins has been slow due to their nonspecific toxicity.

In addition to these antimicrobial peptides, other components of amphibian secretions have been isolated for their anticancer properties, such as the bufadienolide family of compounds derived from the skin glands of Chinese toads (*Bufo melanostictus Schneider* and *Bufo bufo gargarzinas Gantor*). Bufadienolides are a class of C-24 steroids consisting of a characteristic α-pyrone ring at C-17 and include bufalin, cinobufagin, and resibufogenin (F. Li, Wang, He, & Tang, 2008). These compounds have been shown to induce reactive oxygen species-dependent autophagy pathways in a number of cancer cell lines. Yet, their clinical translation has been limited by their cardiotoxicity and low solubility in water, which presents significant formulation challenges (Yin et al., 2012).

Several nanoparticle-based strategies have been employed to enhance the therapeutic potential of amphibian toxins. As with antimicrobial peptides, nanoparticles may sequester

peptide toxin activity until release at a desired therapeutic site (Calderon et al., 2011). For poorly soluble compounds such as bufadienolides, delivery and therapeutic efficacy have been improved following encapsulation in nanoparticles with hydrophobic components, including liposomes (F. Li et al., 2008; F. Li, Yang, Weng, & Tang, 2009) and polymer nanoparticles composed of pluronic polyetherimide (Hu et al., 2014) or mPEG-PLGA-PLL-cRGD block copolymers (Yin et al., 2012).

Bee Venom—The bee venom component most commonly studied for its cytolytic properties is melittin, a peptide derived from the venom of the European honeybee, *Apis mellifera*. Melittin is a cationic amphipathic peptide composed of 26 amino acids and represents approximately 50% of the dry weight of bee venom. Characterized as a host defense peptide, melittin has demonstrated potent antitumor efficacy as a result of its cytolytic activity (Gajski & Garaj-Vrhovac, 2013). The ability of melittin to disrupt lipid membranes arises from oligomerization of melittin peptides to form transmembrane channels which lead to osmotic cell lysis (Tosteson & Tosteson, 1981). Each peptide chain of melittin consists of two α-helical segments which are joined in a "bent rod" configuration due to disruption of the overall helical structure at amino acid positions 10–12 (Bechinger, 1997). Despite its great promise as an antimicrobial and antitumor agent, therapeutic applications of melittin have been severely limited by its nonspecific cytotoxicity and hemolytic activity (Hoskin & Ramamoorthy, 2008).

Nanoparticle delivery systems have been shown to reduce off-target toxicity of melittin and enhance its delivery to therapeutic sites *in vivo*. Soman et al. first demonstrated the ability of melittin to stably bind and insert into the lipid monolayer of perfluorocarbon nanoparticles (Soman et al., 2008). Due to the biophysical properties of the lipid monolayer and the hydrophobicity of the perfluorocarbon core, nanoparticle-bound melittin peptides did not oligomerize or disrupt nanoparticle structure in this construct. In the presence of cell membranes or other lipid nanoparticles, however, formation of a hemifusion stalk between perfluorocarbon nanoparticles and target membranes allowed for melittin transfer and subsequent membrane disruption as shown in Figure 4. Incorporation of melittin into perfluorocarbon nanoparticles significantly reduced systemic toxicity following intravenous administration while retaining antitumor activity against established melanoma tumors and precancerous skin lesions (Soman et al., 2009). N-terminal fusion of melittin with an amphipathic α-helical peptide and subsequent incorporation into lipid nanoparticles also resulted in substantial antitumor activity with reduced nonspecific cytotoxicity (C. Huang et al., 2013)

Scorpion Venom—Scorpion venom contains numerous compounds which may be adapted for cancer therapy. Of these, peptides which block ion channels have been the most heavily studied. The extent of interaction with different types of ion channels depends on the properties of each individual peptide. Longer peptides (60–70 amino acids) with multiple disulfide bridges have been shown to interact with sodium channels while shorter peptides (30–40 amino acids) primarily interact with potassium and chloride channels. The most commonly used scorpion venom toxin is the 36-amino acid peptide chlorotoxin, which is derived from the deathstalker scorpion *Leiurus quinquestriatus* and has historically been

used for the study of voltage-gated chloride channels. In addition to its use as a biological research tool, chlorotoxin has been identified as a potential agent for identification and treatment of gliomas. Chlorotoxin binds reliably and specifically to gliomas and other tumors of neuroectodermal origin (Lyons, O'Neal, & Sontheimer, 2002). Fluorescently labeled chlorotoxin has been shown to stain glioma cells *in vitro*, *in situ*, and in patient tissue samples (Ding, Chua, Bay, & Gopalakrishnakone, 2014). Additionally, synthetic chlorotoxin labeled with radioactive ^{131}I is currently in clinical trials for the treatment of solid tumors and recurrent gliomas. Many different types of nanoparticles have been conjugated to chlorotoxin in order to facilitate delivery of contrast agents (R. Huang et al., 2011; Veiseh et al., 2010) and anticancer therapies (Veiseh et al., 2009, 2010; Hao Wang, Gu, Xiao, Ye, & Xu, 2014) to gliomas. Furthermore, several studies have demonstrated the efficacy of chlorotoxin itself in preventing invasion and migration of glioma cells (Deshane, Garner, & Sontheimer, 2003; Soroceanu, Manning, & Sontheimer, 1999). In addition to chlorotoxin, several other ion channel blockers have been isolated from scorpion venom, including iberiotoxin, margatoxin, and charybdotoxin, all of which could serve as potential therapeutic agents due to their antiproliferative effects on cancer cells.

Snake Venom—Constituents of snake venom have been extensively studied for over 30 years due to their effects on a wide variety of physiological targets. Snake venom peptides and their derivatives have found particular success in the field of cardiovascular medicine. Bradykinin potentiating factors were first isolated from the venom of the lancehead viper *Bothrops jararaca* in the 1960s and demonstrated potent inhibition of angiotensin-converting enzyme (ACE) activity. Structure-function studies of these peptides identified the chemical groups most responsible for this inhibition, eventually leading to the development of the first orally active ACE inhibitor, captopril (Koh & Kini, 2012). ACE inhibitors continue to be extremely popular drugs, with one study reporting that ACE inhibitors are prescribed to 27% of all patients with hypertension (Smith & Ashiya, 2007).

Peptides derived from snake venom have had a substantial impact on the fields of thrombosis and hemostasis due to their pro- and anti-coagulant activity. Factor X activators derived from elapid and viperid venoms, such as Russell's Viper Venom-X, have been primarily used as diagnostic tools in the hematology laboratory for detection of lupus anticoagulants (Takeya et al., 1992; Thiagarajan, Pengo, & Shapiro, 1986). Similarly, prothrombin and factor V activators are beneficial for diagnostic use due to the high specificity of venom components for their substrates. For instance, factor V remains the only known substrate for Russell's Viper Venom-V, allowing this compound to be developed into a specific tool for detection of factor V (Esmon & Jackson, 1973; McCleary & Kini, 2013). Ecarin, an enzyme derived from the venom of the saw-scaled viper *Echis carinatus* promotes conversion of prothrombin to meizothrombin but is inhibited by hirudin, thereby serving as the basis for a clotting time assay for patients treated with hirudin (Nowak, 2003). Additionally, the class of enzymes known as snake venom thrombin-like enzymes (SVTLEs) has found use in diagnostic labs to defibrinogenate blood samples, as addition of SVTLEs results in formation of loose fibrin clots due to cleavage at only one of the two fibrinogen chains (Koh & Kini, 2012). Other venom components have been utilized for their procoagulant effects on platelets, where snaclec (snake C-type lectin-like) toxins have

demonstrated platelet activation through binding of von Willebrand factor (e.g., botrocetin, bitiscetin) or direct binding to platelet glycoprotein receptors (e.g., convulxin) (Sajevic, Leonardi, & Križaj, 2011). Platelet aggregation has also been shown to occur through activation of protease activated receptors on platelets by snake venom serine proteases such as thrombocytin (McCleary & Kini, 2013).

The major classes of anticoagulants present in snake venom include enzymes such as phospholipase A_2 (Carredano et al., 1998) and peptides such as snaclecs and "three finger toxins" (Kini, 2006). Snaclecs, including anticoagulation factor I, bind to coagulation factors IX and X, resulting in inhibition of clotting as measured by activated partial thromboplastin time (aPTT), prothrombin time (PTT), and thrombin time (TT) (Zhang et al., 2012). Similarly, three-finger toxins, which consist of 4–5 disulfide bridges and three β-stranded loops (Kini, 2006) , have been studied for their anticoagulant effects on the extrinsic pathway of the clotting cascade (Kini, Haar, & Evans, 1988) and factor VIIa inhibition (Banerjee, Mizuguchi, Iwanaga, & Kini, 2005).

Outside of the cardiovascular field, peptides derived from snake venom have been studied as anticancer therapies due to their proapoptotic properties. To this end, silica nanoparticles bearing venom from the black snake *Walterinnesia aegyptia* have been shown to enhance function of normal lymphocytes (Badr, Al-Sadoon, El-Toni, & Daghestani, 2012), as well as induce apoptosis in human prostate cancer cells (Badr, Al-Sadoon, Rabah, & Sayed, 2013) and mouse models of multiple myeloma (Al-Sadoon, Rabah, & Badr, 2013).

Conclusion

Natural peptides possess unique biological, chemical, and physical properties which underlie their application as therapeutic agents. The use of natural peptides in conjunction with nanoparticle delivery systems holds great promise for the development of new therapeutic formulations as well as novel platforms for the delivery of various cargoes.

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A

Aqueous
Core

Liposome

B

Perfluorocarbon
Core

Perfluorocarbon
Nanoparticle

C

Free Polymer Chains

Aggregation

Polymer
Nanoparticle
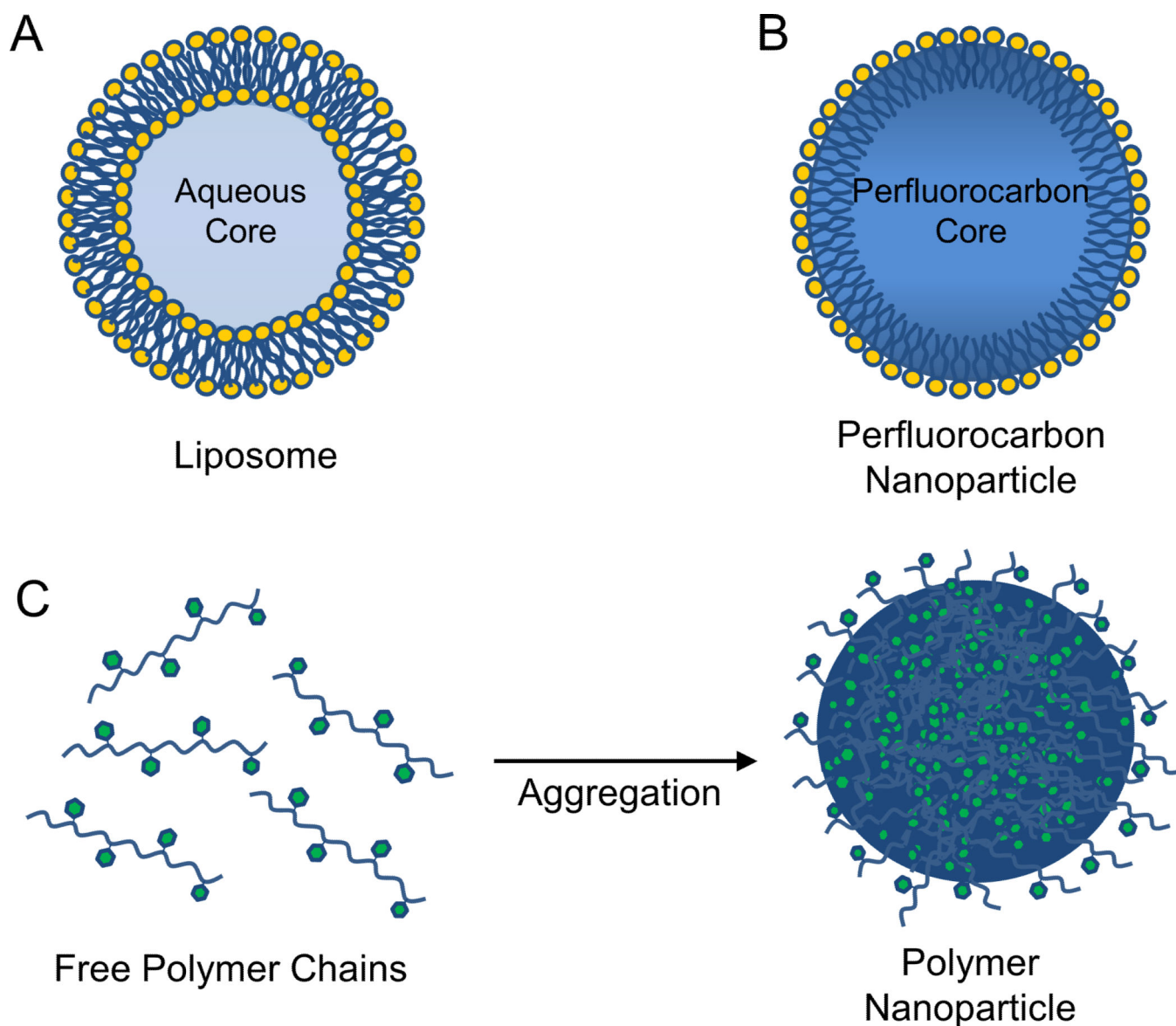
Figure 1.
Structure of lipid and polymer nanoparticles. (**A**) Liposomes consist of a phospholipid bilayer surrounding an aqueous core. (**B**) Perfluorocarbon nanoparticles consist of a phospholipid monolayer surrounding a hydrophobic perfluorocarbon core. (**C**) Polymer nanoparticles are often formed by aggregation of hydrophobic polymer chains in an aqueous environment.
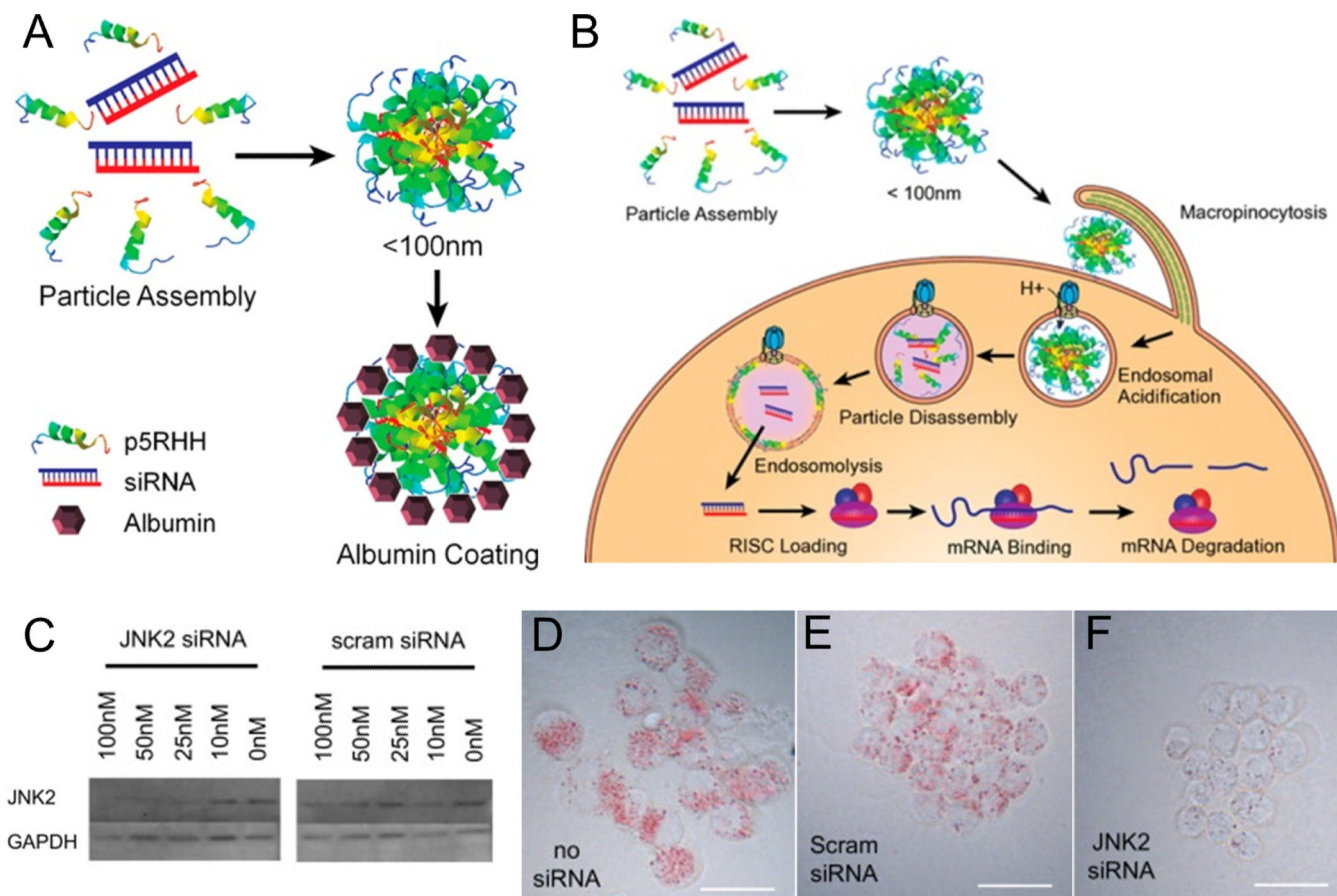
Figure 2.
Self-assembly and efficacy of p5RHH-siRNA nanocomplexes. (**A**) Schematic diagram of p5RHH and siRNA complexation. (**B**) Schematic diagram of p5RHH-siRNA complex internalization and disassembly following endosomal acidification. (**C**) Application of p5RHH-siRNA complexes containing JNK2 siRNA lowered JNK2 expression in a macrophage cell line. (**D, E**, and **F**) Application of p5RHH-siRNA complexes containing JNK2 siRNA reduced foam cell formation following exposure to acetylated LDL. Panels **A** and **B** adapted with permission from Hou, K. K., Pan, H., Ratner, L., Schlesinger, P. H., & Wickline, S. A. (2013). Mechanisms of Nanoparticle-Mediated siRNA Transfection by Melittin-Derived Peptides. *ACS Nano, 7*(10), 8605–8615. Copyright 2013 American Chemical Society. Panels **C, D, E**, and **F** adapted with permission from Hou, K. K., Pan, H., Lanza, G. M., & Wickline, S. A. (2013). Melittin derived peptides for nanoparticle based siRNA transfection. *Biomaterials 34*(12), 3110–3119.Copyright 2013 Elsevier.
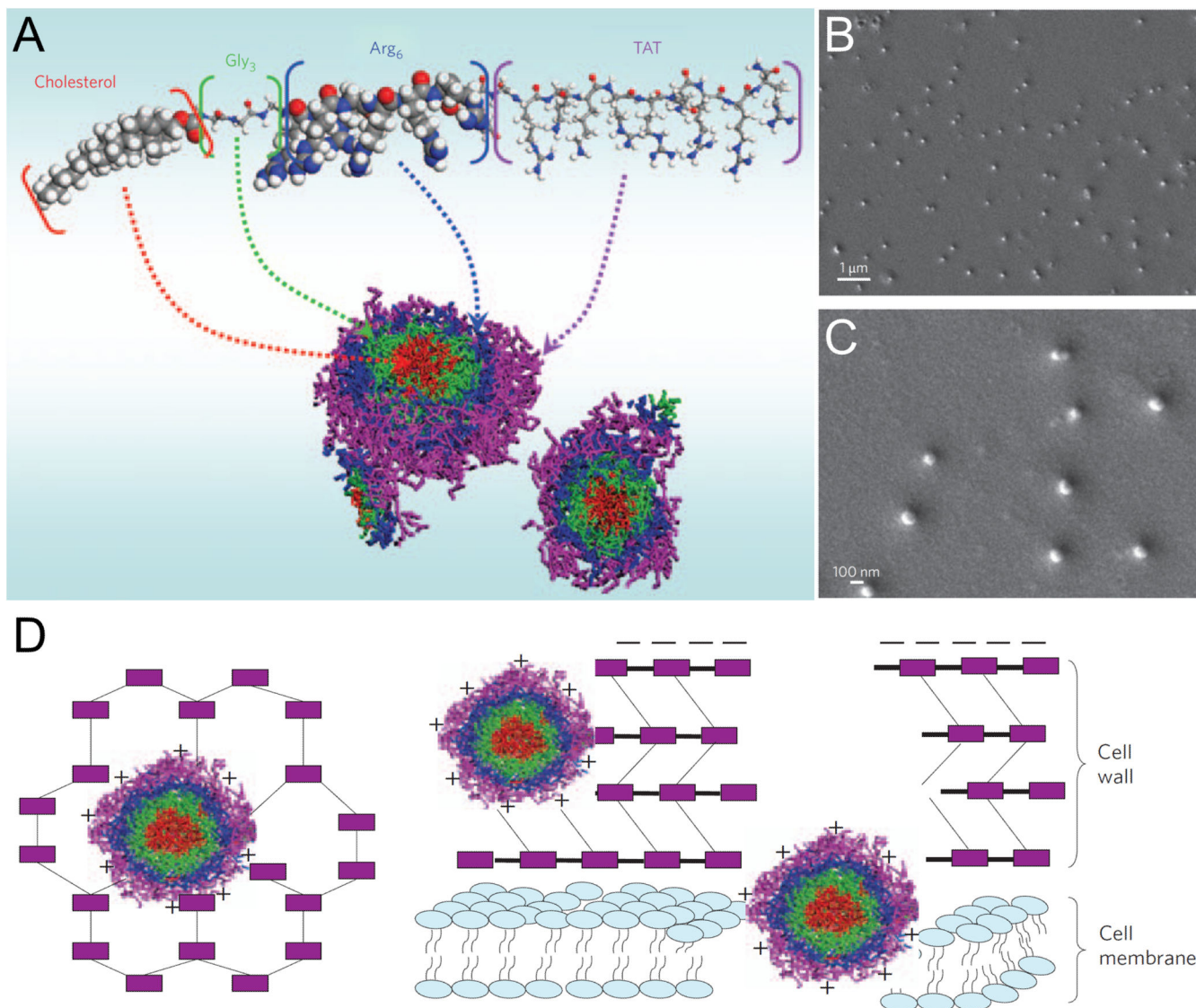
Figure 3.
Self-assembly and mechanism of action of CG_3R_6TAT nanoparticles. (**A**) Schematic diagram of CG_3R_6TAT self-assembly into nanoparticles with core-shell morphology. (**B** and **C**) Scanning electron micrographs of CG_3R_6TAT nanoparticles. (**D**) Schematic diagram of CG_3R_6TAT nanoparticle antimicrobial activity. Electrostatic interaction with anionic cellular components leads to cell wall and cell membrane disruption. All panels adapted with permission from Liu, L., Xu, K., Wang, H., Tan, P. K. J., Fan, W., Venkatraman, S. S., Li, L., & Yang, Y.-Y. (2009). Self-assembled cationic peptide nanoparticles as an efficient antimicrobial agent. *Nature Nanotechnology, 4*(7), 457–463. Copyright 2009 Nature Publishing Group.
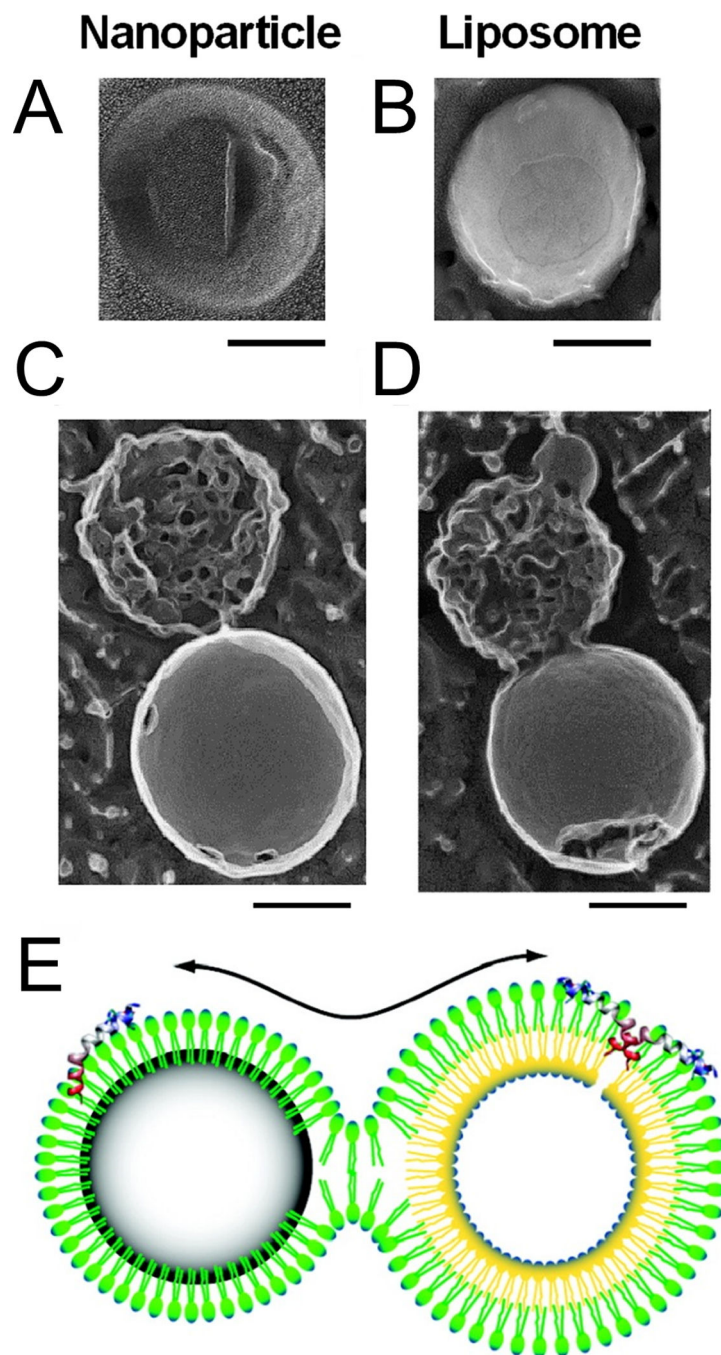
Nanoparticle   Liposome

Figure 4.
Interaction of melittin-loaded perfluorocarbon nanoparticles with liposomes. (**A**) Deep etch platinum replica of a perfluorocarbon nanoparticle. (**B**) Deep etch platinum replica of a liposome. (**C** and **D**) Deep etch platinum replicas of perfluorocarbon nanoparticle-liposome complexes. Scale bars correspond to 100 nm. (**E**) Schematic diagram of hemifusion stalk formation and melittin transfer between perfluorocarbon nanoparticles and liposomes. All panels adapted with permission from Soman, N. R., Lanza, G. M., Heuser, J. M., Schlesinger, P. H., & Wickline, S. A. (2008). Synthesis and Characterization of Stable

Fluorocarbon Nanostructures as Drug Delivery Vehicles for Cytolytic Peptides. *Nano Letters, 8*(4), 1131–1136. Copyright 2008 American Chemical Society.

Table 1

Natural peptides used for nanoparticle and drug design

Peptide	Sequence	Origin
Tat Peptide	YGRKKRRQRRR	HIV-1 *trans*-activator of transcription (Tat) protein
Penetratin	RQIKIWFQNRRMKWKK	*Drosophila* Antennapedia protein
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL	Neuropeptide galanin / Wasp venom mastoparan
MPG	GALFLGFLGAAGSTMGAWSQPKKKRKV	HIV-1 glycoprotein 41 / Simian virus 40 large T antigen
Pep-1	KETWWETWWTEWSQPKKKRKV	HIV-1 reverse transcriptase / Simian virus 40 large T antigen
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	European honeybee venom
Magainin II	GIGKWLHSAKKFGKAFVGEIMNS	African clawed frog secretion
Cecropin A	KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK	Cecropia moth hemolymph
Buforin II	TRSSRAGLQFPVGRVHRLLRK	Asian toad stomach tissue
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTESC	Human cathelicidin
Chlorotoxin	MCMPCFTTDHQMARKCDDCCGGKGRGKCYGPQCLCR	Deathstalker scorpion venom