International Journal of
Molecular Sciences

MDPI

Review

Peptide-Assisted Nucleic Acid Delivery Systems on the Rise

Shabnam Tarvirdipour [1,2], Michal Skowicki [1,3], Cora-Ann Schoenenberger [1,3,*] and Cornelia G. Palivan [1,3,*]

[1] Department of Chemistry, University of Basel, Mattenstrasse 24a, 4058 Basel, Switzerland; shabnam.tarvirdipour@unibas.ch (S.T.); michaljerzy.skowicki@unibas.ch (M.S.)
[2] Department of Biosystem Science and Engineering, ETH Zurich, Mattenstrasse 26, 4058 Basel, Switzerland
[3] NCCR-Molecular Systems Engineering, BPR1095, Mattenstrasse 24a, 4058 Basel, Switzerland
* Correspondence: cora-ann.schoenenberger@unibas.ch (C.-A.S.); cornelia.palivan@unibas.ch (C.G.P.)

Abstract: Concerns associated with nanocarriers' therapeutic efficacy and side effects have led to the development of strategies to advance them into targeted and responsive delivery systems. Owing to their bioactivity and biocompatibility, peptides play a key role in these strategies and, thus, have been extensively studied in nanomedicine. Peptide-based nanocarriers, in particular, have burgeoned with advances in purely peptidic structures and in combinations of peptides, both native and modified, with polymers, lipids, and inorganic nanoparticles. In this review, we summarize advances on peptides promoting gene delivery systems. The efficacy of nucleic acid therapies largely depends on cell internalization and the delivery to subcellular organelles. Hence, the review focuses on nanocarriers where peptides are pivotal in ferrying nucleic acids to their site of action, with a special emphasis on peptides that assist anionic, water-soluble nucleic acids in crossing the membrane barriers they encounter on their way to efficient function. In a second part, we address how peptides advance nanoassembly delivery tools, such that they navigate delivery barriers and release their nucleic acid cargo at specific sites in a controlled fashion.

Keywords: amphiphilic peptides; non-viral gene delivery; nanocarrier; peptide self-assemblies; stimuli responsive

Citation: Tarvirdipour, S.; Skowicki, M.; Schoenenberger, C.-A.; Palivan, C.G. Peptide-Assisted Nucleic Acid Delivery Systems on the Rise. *Int. J. Mol. Sci.* **2021**, *22*, 9092. https://doi.org/10.3390/ijms22169092

Academic Editors: Nunzianna Doti and Menotti Ruvo

Received: 20 July 2021
Accepted: 19 August 2021
Published: 23 August 2021

1. Introduction

Introducing exogenous nucleic acids into human target cells has been receiving a great deal of attention for the treatment of several human diseases, in particular cancer and other genetic disorders. Quite recently, a new treatment involving gene editing CRISPER has made a mark by using mRNA encoding Cas [1,2]. In face of the worldwide coronavirus pandemic, mRNA has moved into the limelight as vaccine and many companies are working on other mRNA vaccines and therapeutics [3,4]. Both vaccines and disease intervention involve delivering nucleic acids to intracellular locations on a path strewn with obstacles. To ultimately accomplish modification of protein expression by replacing or adding missing or defective genes, regulating gene expression at the RNA level (e.g., gene silencing by RNA interference, modification of RNA processing), controlling microRNA activity or by genome editing and reprogramming of cells, nucleic acids face a number of challenging barriers. Hence, despite a broad range of possible therapeutic approaches, the clinical success of gene therapy has yet to meet the expectations. The lack of efficacy and issues with clinical safety, in particular with viral vectors, which make up about 70% of vectors used in gene therapy, are the main reasons gene delivery systems fail in clinical trials [5,6]. This has led to the emergence of non-viral vector systems, such as liposomes and polymer supramolecular assemblies with better biological safety. However, their efficacy is predominantly hampered by insufficient localization of the therapeutic agents at the site of interest, both at the extracellular and intracellular level [7]. Owing to their remarkable potency, selectivity and low toxicity, peptides offer ideal alternatives to overcome these hurdles [8]. In addition, advancements in nanosystems continue to open new avenues for

an efficient delivery of therapeutics and, thus, nanotechnology has become a favored tool in medicine [7].

Nanocarriers based on their size, shape, charge, and surface chemistry are internalized by target cells through different pathways including clathrin-mediated endocytosis, caveolae- or cholesterol-mediated endocytosis, phagocytosis, and macropinocytosis [9,10]. After entering cells by endocytosis, nanocarriers usually remain sequestered in corresponding transport vesicles and their fate depends on the endocytic pathway but also on the physicochemical properties of the nanocarriers. Endosomal sequestration consists of multiple membrane fusions, in which the endocytic vesicles sequentially merge with early and late endosomes, proceeding all the way to the lysosomal compartment [10]. A constant decrease in intravesicular pH and increase in digestive enzymatic content throughout this pathway have a major impact on the stability of payloads and, subsequently, on efficacy [10,11]. These limitations have led to the search for strategies that can properly protect the macromolecular drugs from degradation and specifically target the major subcellular compartments. Furthermore, a boost of discovery research for the better understanding of intracellular trafficking routes highlight the need for carriers that overcome the barriers associated with the delivery to the intracellular site of action [11].

The major shortcomings of most commonly used non-viral nucleic acid delivery systems, such as lipoplexes and polyplexes include nonspecific distribution, inefficient cytoplasmic delivery, and organelle targeting. In contrast, peptide-based nanocarriers, e.g., peptide nanoparticles, also called peptiplexes, or peptidic multicompartment micelles, and nano-assemblies equipped with peptides hold great promise as delivery platforms, since they can be tweaked to facilitate penetration of cell membranes and to localize to distinct subcellular compartments. In addition, peptides are easy to synthesize with a desired bioactivity, and, by multivalent presence, endow the nanocarrier with high avidity for the target [10,12]. Owing to the highly specific targeting capacity of corresponding peptides, therapeutic nanocarriers are able to pass through the cell membrane and reach the specific tissue and cells which results in enhanced intracellular distribution and extended therapeutic window [13]. Furthermore, smart delivery systems are promising options to provide solutions related to uncontrolled release of payloads: besides a biocompatible nanocarrier and suitable targeting moieties, these platforms include stimulus-responsive elements which endow them with triggered cargo release [14].

The concept of using peptides as targeting moieties for therapeutic and diagnostic purposes has created new avenues for modern pharmaceutical industries [13,15]. Although clinical progress in the application of peptides, alone or combined with nano-assemblies, is slowly moving forward, large investments and wide-ranging research efforts confirm their promising potential as a delivery platform for therapeutic systems. Increased interest in smart nanocarrier design with particular focus on, but not limited to, cancer therapy with the aim of precision medicine application has boosted this unique class of pharmaceutical compounds into high demand [16–18].

In this review, we discuss various types of membrane active and stimuli responsive peptides with regard to their role in refining different nanocarriers for gene delivery applications. As peptides take center stage, we do not cover predominantly lipidic nor inorganic nanoparticle gene delivery systems. We describe properties of peptides that promote site-specific localization of nucleic acids and of peptide-based nano-assemblies. Then, we lay the emphasis on peptide designs that confer stimuli-responsiveness upon nanosystems with the aim to control payload release. Targeting, controlling, and stimuli-responsive peptides advance nanosystems from non-specific carriers of nucleic acids to smart site-specific gene delivery systems.

2. Peptide-Guided Delivery of Nucleic Acids across Biological Barriers

Membrane active peptides interact with cellular membranes by traversing them, disrupting them or by residing at the membrane interface and fusing with them [19]. They are known to overcome site-specific delivery barriers and facilitate intracellular delivery

of various bioactive cargos with low cytotoxicity [19,20]. Although there is a wide variety of membrane-active peptides, here we mainly discuss peptides for targeting nucleic acid delivery systems to specific cells and tissues, and peptides that assist in the delivery of nanocarriers across membrane barriers, such as cell penetrating peptides (CPPs), peptides facilitating endosomal escape and those that target nanocarriers to subcellular organelles (Figure 1).

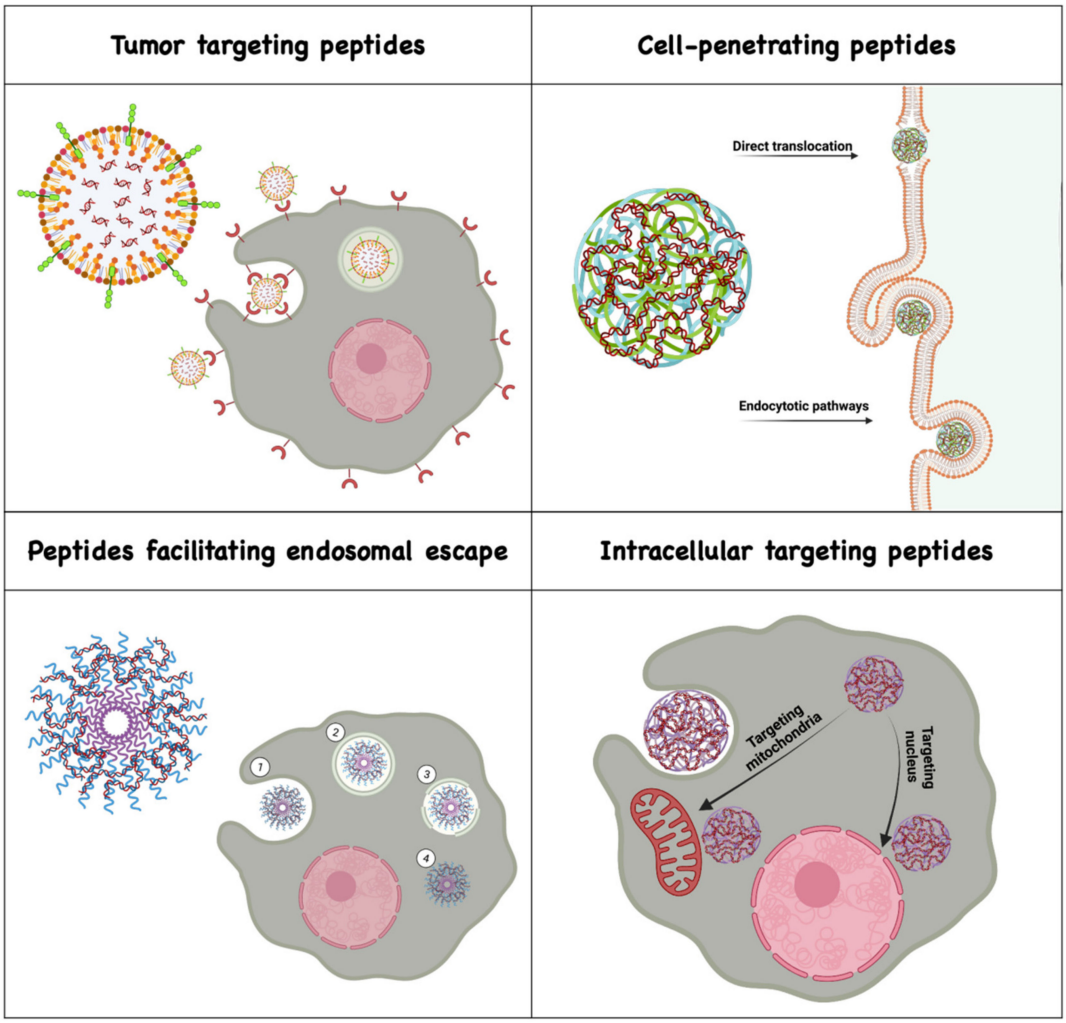

Figure 1. Classes of membrane active peptides facilitating the delivery of nucleic acid across biological barriers. Created with BioRender.com (Access to BioRender: June–July).

2.1. Tumor-Targeting Peptides

The ability of peptides to mediate translocation across membranes, traffic to desired sites, as well as executing many fundamental cellular functions made them promising candidates for targeting [21]. Owing to the high mortality related to cancer, substantial research investments have been made over the past decades in order to develop specific cancer diagnostics and treatments that improve survival rate [22]. The aberrant proliferation of tumor cells, accompanied by the up-regulation of their molecular markers result in high levels of specific receptors in the tumor and its microenvironment [23]. Thus, tumor-targeted delivery methods incorporate peptides or antibodies that are selective to the receptors overexpressed on the tumors [24]. Selective targeting of these tumor-associated markers promises the accurate targeting of signaling pathways that are dysregulated in the tumor [25].

Although the use of antibodies to target tumors has become highly successful both in tumor diagnosis and therapy, some deficiencies associated with antibodies, such as inadequate pharmacokinetics and limited tissue accessibility, as well as impaired interactions with the immune system limit their clinical application. Compared to antibodies and other tumor-targeting ligands, peptides offer better cell or tissue penetration, high affinity and targeting specificity, low immunogenicity, high stability, and improved pharmacokinetics by chemical modifications [26]. Tumor-targeting peptides, usually comprising less than 50 amino acids, are synthesized naturally or artificially [27,28]. For example, peptide sequences containing an arginine-glycine-aspartic acid (RGD) motif are among the most prominent targeting moieties for non-viral delivery systems [29]. The strong affinity of the RGD motif for integrin receptors expressed on vascular endothelial cells and overexpressed on many cancer cells [30] facilitates cell attachment and uptake of nanocarriers by receptor-mediated endocytosis (Figure 2) [31].

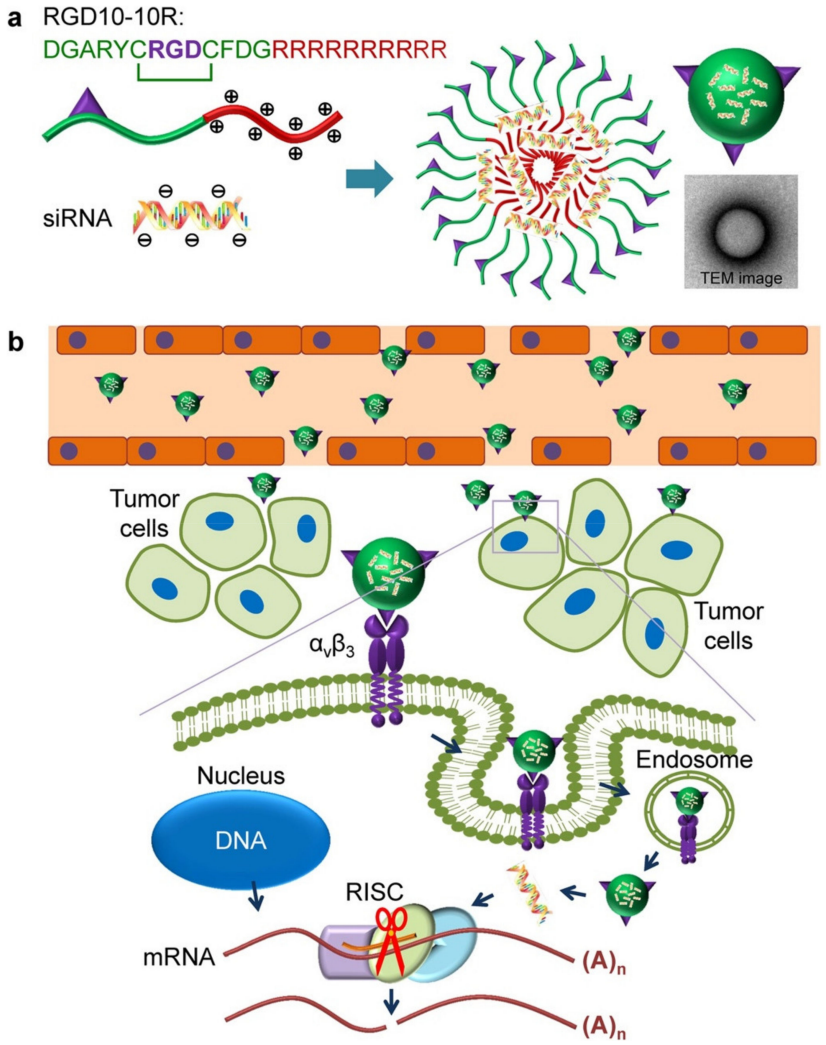

Figure 2. Schematic representation of (**a**) fabrication of the RGD10-10R/siRNA complex, (**b**) tumor-targeted siRNA delivery involving ligand/receptor interactions. siRNAs accumulated in the tumor tissue and then entered the tumor cells in a receptor (αvβ3)-mediated endocytosis (RME) manner in vitro. After being internalized by cells, peptide/siRNA complexes escaped from the endosomes/lysosomes. Then, siRNAs were released from the complexes and loaded by RNA-induced silencing complex (RISC). Targeted messenger RNA complementary to the guide strand (antisense strand) of siRNA was selected and cleaved by argonaute protein. Reprinted with permission from [31]. Copyright 2015 Springer Nature.

Likewise, the synthetic nonapeptide LyP-1 is an example of a tumor targeting peptide that can selectively bind to its primary receptor p32 protein overexpressed in various tumor-associated cells and atherosclerotic plaque macrophages [32]. Binding leads to proteolytic cleavage of LyP-1 into a truncated version whose exposed C-terminal CendR motif becomes active and triggers binding to NRP1 and/or NRP2 cell surface receptors [32,33]. This interaction promotes cellular internalization of LyP-1 and its bioconjugates. NRP1/2 also mediates transfer to the nucleus, which makes LyP 1-based delivery systems more effective in imaging and treatment of diseases [34]. An overview of different tumor-targeting peptides developed for cancer gene therapy is presented in Table 1.

Table 1. Examples of peptides used for targeting in cancer gene therapy.

Peptide Name	Cargo	Cancer Type	Ref.
RGD	siRNA	breast	[35]
cRGD	siRNA	brain	[36]
	siRNA	skin	[37]
iRGD	siRNA	pancreatic	[38]
	siRNA	lung	[39]
RGDfC	siRNA and doxorubicin	liver	[40]
CRGDK	siRNA and BAplatin	breast	[41]
CGKRK	siRNA	breast and brain	[42]
KTLLPTP	siRNA and paclitaxel	pancreatic	[43]
HAIYPRH	siRNA and doxorubicin	breast	[44]
LyP-1 and iRGD	siRNA	ovarian	[45]
YHWYGYTPQNVI	siRNA	liver	[46]
T7	pDNA	bone	[47]

2.2. Cell-Penetrating Peptides

Cell-penetrating peptides (CPPs) are short peptides (less than 30 amino acids) derived from naturally occurring proteins, designed de novo or a combination of both [48]. CPPs by virtue of their ability to permeate the cell membrane in an innocuous manner provided a means for successful cellular entry and intracellular trafficking of a wide variety of cargos including nucleic acids (Figure 3) [49–53]. In addition to sequence length, charge and amphipathicity are the main structural parameters determining internalization but also cargo interactions. Penetration of nucleic acids across the cell membranes is a key step in gene delivery and paves the way for an efficient gene therapy [52]. Nucleic acids can be conjugated to CPPs, either by non-covalent complex formation or by covalent bonds [54]. CPPs promote the intracellular distribution of these membrane-impermeable therapeutic molecules without destroying the integrity of cellular membranes and, thus, widen the therapeutic window of cargos [13].

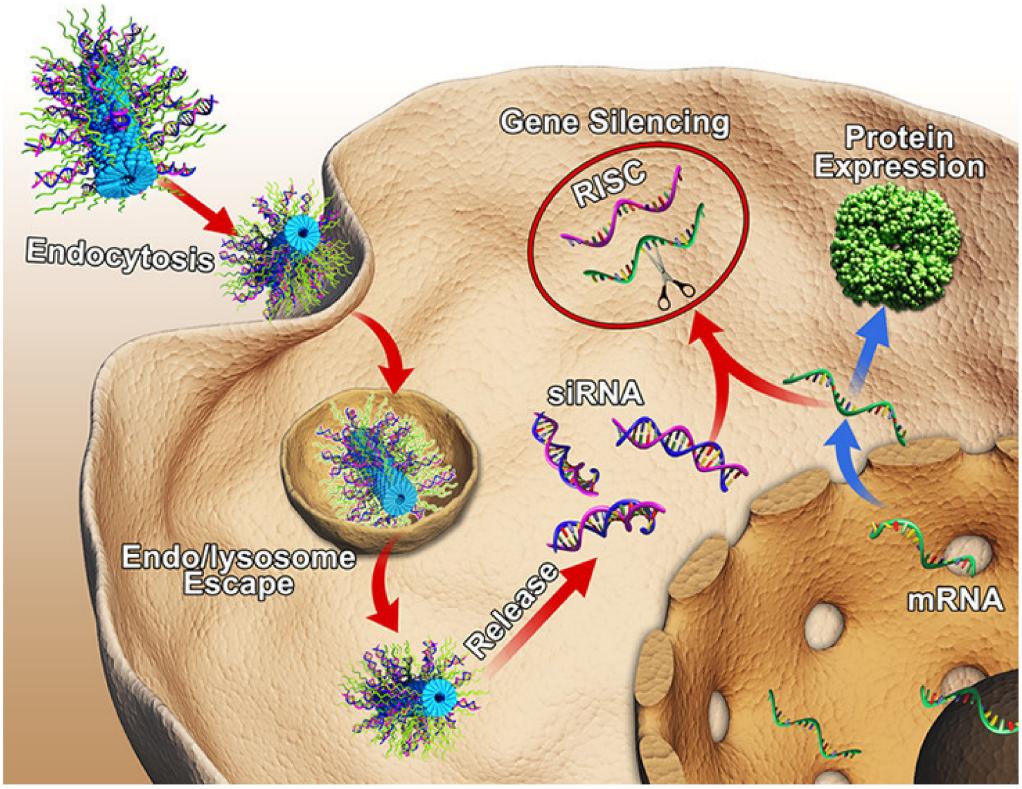

Figure 3. Schematic illustration of cell penetrating TAT peptides complexed with siRNA and integrated into modified tobacco mosaic virus (TMV) for virus-inspired gene silencing. Reprinted with permission from [52]. Copyright 2018 American Chemical Society.

CPPs can be classified according to their physicochemical properties as cationic, amphipathic, and hydrophobic, which largely impacts the type of cell-membrane interactions and uptake mechanism [55]. Extensive literature is available on the structure–activity relationship of CPPs [56–59]. Examples of CPPs classified according to their physicochemical properties and the genetic cargo they delivered are summarized in Table 2.

Table 2. CPP classification based on physicochemical properties.

Cell-Penetrating Peptides								
Cationic			**Amphipathic**			**Hydrophobic**		
Name(origin)	**Cargo**	**Ref.**	**Name(origin)**	**Cargo**	**Ref.**	**Name(origin)**	**Cargo**	**Ref.**
Diatos Peptide Vectors (DPV)	siRNA	[60]	MPG	pDNA siRNA	[61,62]	C105Y	pDNA	[63–66]
HIV-1 twinarginine translocation (TAT)	pDNA siRNA	[67–74]	Transportan	pDNA siRNA	[72,75,76]	K-FGF	pDNA	[77]
arginine-rich peptides	pDNA siRNA	[78–82]	NickFect (NF)	pDNA siRNA	[83–86]	Bip	pDNA	[87]
Polyarginine	pDNA	[75,76,88–90]	PepFect (PF)	pDNA mRNA	[91,92]	Melittin-derived peptides	siRNA	[93]
Penetratin	pDNA	[94–96]	MAP	siRNA	[97]			
L5a	pDNA	[98,99]	Crotamine	pDNA	[100–102]			
			VP22	pDNA	[103,104]			
Protamine	pDNA mRNA	[105–109]	Antennapedia (Antp)	AON siRNA	[110,111]			
			Pep-1	pDNA	[112,113]			
			CADY	siRNA	[114–116]			
			FGF	pDNA	[77]			
			pVEC	pDNA	[117,118]			

Cationic CPPs show a high affinity for negatively charged cell membranes because of electrostatic interactions and, thus, internalize into the cell through a receptor-independent mechanism. The key factors determining the activity of cationic CPPs are the number and position of positively charged amino acids in their structure [57]. TAT and penetratin, the first cationic CPPs discovered, have been widely used to promote cellular uptake and transfection efficiency of various lipid-, polymer-, and peptide-based nanocarriers [119–121]. Accordingly, several artificial homopolymers of arginine and lysine peptides have been developed to effectively translocate cargo across the membrane [122,123]. Notably, the rate of cell uptake and subsequently transfection efficiency was higher for arginine-rich peptides compared to polylysines [123–125].

Although most naturally occurring CPPs are cationic, the major class of CPPs is amphipathic [48]. Amphipathic CPPs consist of polar and non-polar (rich in hydrophobic) amino acid regions that are able to fold into α-helical and β-sheet-like structures. The secondary structure might change in response to different physiological conditions which, in turn, affects their penetration ability [57]. Prominent representatives of amphipathic CPPs are various variants of N-Methylpurine DNA Glycosylase or MPG, where amphiphilicity is a leading factor for their translocation across the membrane [126]. MPGs undergo a conformational transition from unordered into a folded state upon their interaction with membrane phospholipids mediated by polar residues. The resulting β-sheet conformation governed by the hydrophobic domain of MPG lead to transient pore-formation in the cell membrane, which in turn enable the MPG/cargo complexes direct penetration across the membrane independent of endocytosis [126,127]. In addition to MPGs' function in promoting cellular internalization, it is well known for its strong electrostatic interactions with oligonucleotides [128]. Consequently, MPG family members form stable noncovalent nanocomplexes with nucleic acids that enter cells independently of the endosomal pathway. Accordingly, MPG has shown to efficiently deliver small interfering RNA (siRNA) and plasmid DNA (pDNA) into cultured cell lines [129]. Transportan and its analogs NickFect and PepFect are other examples of amphipathic peptides that can condense pDNA and siRNA into stable nanocomplexes [130–132]. Although their hydrophobicity appears to be responsible for the nanocomplexes' stability, the pH-induced change of their charge plays a key role in promoting oligonucleotide condensation and high delivery efficiency.

Hydrophobic CPPs with low positive or negative net charge are less common and their uptake mechanism is not well understood. For example, natural C105Y, K-FGF, and Bip peptide belong to this group and their non-polar amino acids' affinity to the hydrophobic domain of cell membranes mediate their translocation [48].

2.3. Peptides Facilitating Endosomal Escape

Endosomal escape is a crucial step in improving intracellular delivery and efficiency of nucleic acids [133]. Following endocytosis as the major uptake route for many peptide-based nanocarriers, most internalized nanocarriers significantly suffer from their interaction with endosomal membrane which leads to their entrapment and eventually their enzymatic degradation in the lysosomal compartment [134,135]. Peptides are the most promising candidates for promoting endosomal escape [136,137]. In particular, pH-sensitive peptides that at physiological pH adopt a random coil structure, transform to an α-helical conformation able to induce membrane pore formation in the acidic environment of endosomes [138]. Similarly, studies on viruses escaping the lysosome have shown that some viral peptides change from a hydrophilic ring structure to a hydrophobic spiral structure which will target the core of the bilayer and destroy the stability of the membrane [139]. Peptide development aims at facilitating escape from the endosome via pore formation, the "proton sponge effect", or conformational changes.

2.3.1. Fusogenic Peptides

Fusogenic peptides (FPs) are short peptides with the potential to promote membrane destabilization and delivery of nucleic acids to the cytosol and/or the nucleus [140]. Fu-

sogenic peptides consist of hydrophilic and hydrophobic domains that are able to form helical structures at endosomal pH. This allows for direct engagement with the endosomal membrane upon which further energetically favorable conformational changes induce pore formation in the membrane. Disruption of the bilayer eventually leads to endosomal escape of nanocarriers equipped with FPs and release of cargos to the cytoplasm (Figure 4). Overcoming the endosomal membrane barrier presents an important role in facilitating nucleic acids localization to distinct subcellular compartments as their site of action [54]. However, before integrating a fusogenic peptide into gene delivery systems, the cellular uptake mechanism should be considered. Since the fusogenic activity of these peptides is due to a pH-dependent shift in conformation, non-acidic endocytotic pathways, such as caveolae-mediated endocytosis and macropinocytosis will revoke their membrane lytic activity [141].

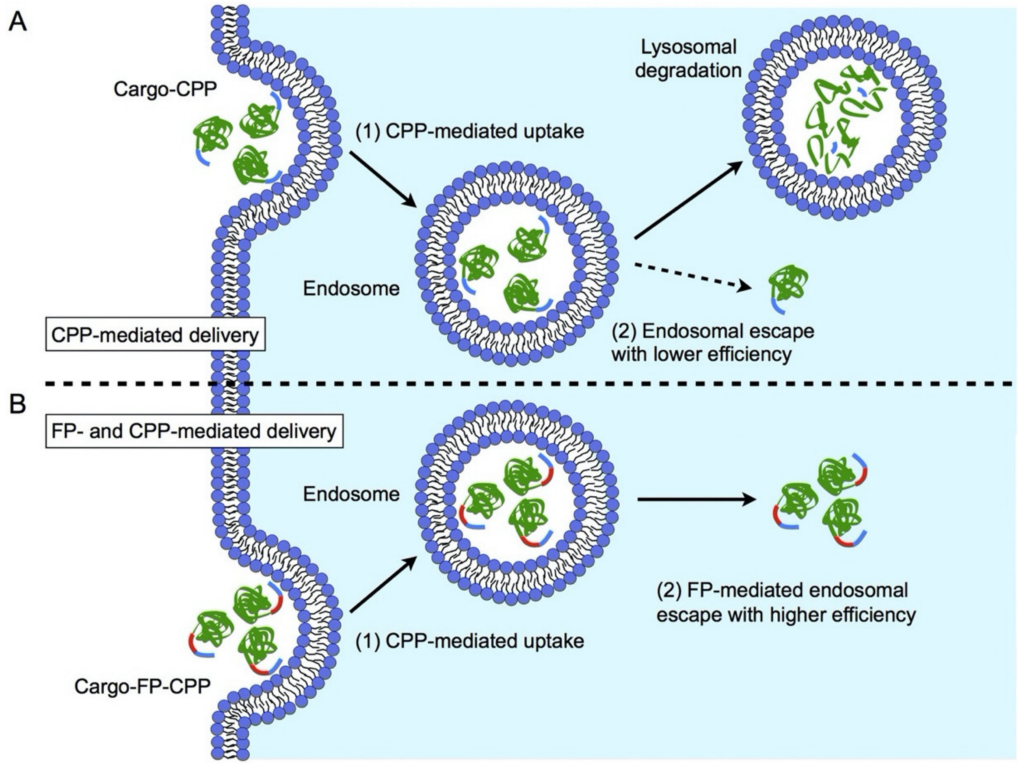

Figure 4. Schematic representation of FP and CPP-mediated delivery. (**A**) A conventional CPP-mediated delivery. (1) (**A**) cationic CPP (blue) interacts electrostatically with the anionic cell-surface, and a CPP-fused cargo (green) is internalized into the endosome by endocytosis. (2) Because the endosomal escape efficiency of CPP is low, the cargo-CPP is subjected to lysosomal degradation. (**B**) FP- and CPP-mediated delivery. (1) An FP (red)- and CPP-fused cargo is internalized into the endosome by endocytosis. (2) Because the efficiency of FP-mediated endosomal escape is relatively high, the cargo-FP-CPP is efficiently transferred from the endosome to the cytoplasm. Reprinted with permission from [142]. Copyright 2017 Elsevier.

Fusogenic peptides are either derived from the transduction domain of proteins that interact with cell membranes such as HA2, INF7, and melittin or are synthetic amphipathic peptides that can penetrate membranes [141,143].

Wild-type HA2(1–23) peptide and a glutamic acid-enriched analogue (INF7) from influenza virus hemagglutinin are the oldest and best studied fusogenic peptides used for gene delivery [144–149]. These peptides, based on the protonation of their acidic residues upon a decrease in pH, assume a helix structure and consequently promote the endosomal escape, which, in turn, results in enhanced transfection efficiency [150,151].

Melittin, a cationic amphipathic peptide composed of 26 amino acids, is derived from the venom of the honey bee Apis mellifera. Melittin with its predominantly hydrophobic 20 N-terminal amino acids and hydrophilic C-terminus acts mainly like a natural detergent on the membrane and is well known for its cytolytic activity [152]. Oligomerization of this peptide results in the formation of transmembrane channels which lead to osmotic cell lysis [153]. Owing to its cytotoxicity, the use of melittin as an agent to promote gene delivery in transfected mammalian cells is limited. More recently, less toxic melittin analogues that retained their ability to escape from the endosome were shown to enhance the efficiency of non-viral gene delivery systems [154–156].

A number of pH-responsive synthetic amphipathic peptides mimic the fusogenic activity of virus-derived peptides. The most prominent representatives consist of nonpolar alanine-leucine-alanine repeating units with considerable repetitive content of either glutamic acid, lysine, or arginine, and are named GALA, KALA, or RALA, respectively [157–161]. Likewise, upon protonation at endosomal pH (5.0), they assume an amphipathic α-helical conformation which is associated with a significant affinity for binding to phospholipid membranes. As a consequence, pore formation, membrane fusion, and/or lysis are induced. Their membrane lytic activity explains extensive utilization of these fusogenic peptide in modulating non-viral gene delivery systems [76,162–166]. Similar to GALA, JTS-1, a negatively charged amphipathic peptide with strong nonpolar amino acids in the hydrophobic domain and glutamic acid residues in the hydrophilic domain is able to form an α-helical structure [167]. Owing to the endosomolytic capacity of JTS-1-modified carriers, improved transfection activity was reported in several studies [168,169].

Taking advantage of the fusogenic properties of peptides, alone or in combination with other advantageous attributes, promotes the efficacy of peptide-based nanocarriers for traversing membranes and, thereby, improves their therapeutic effects. Yet, there is a strong need for systematic study of different fusogenic peptides under similar conditions in order to elucidate the mechanisms and pin down the parameters that ultimately will maximize therapeutic outcome. This comprehensive comparison of fusogenic peptides will allow for designing a robust, widely applicable delivery system.

2.3.2. Histidine-Rich Peptides

The combination of being able to condense nucleic acids and at the same time promote endosomal escape spurred efforts to incorporate histidine-rich amphipathic peptides into various gene delivery systems [170]. As described for fusogenic peptides, histidine residues that become protonated during acidification of the endosome interact with negatively charged membrane lipids (Figure 5) and destabilize the membrane [171]. Histidylation of different non-viral vectors among which polylysine was the first example, was found to increase the buffering capacity of vectors [172]. Substituting several lysines with histidines turns polylysine into a successful gene delivery vector with enhanced transfection efficiency [173–178].

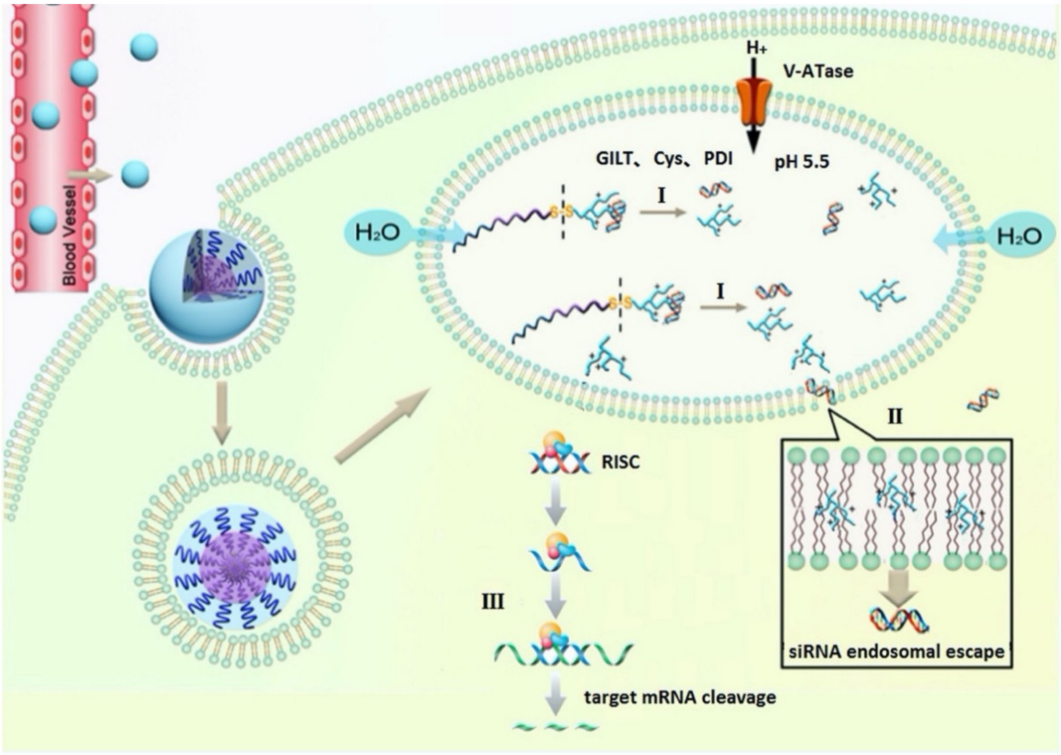

Figure 5. Schematic illustration of the intracellular trafficking of mPEG-b-PLA-Phis-ssPEI and siRNA complexes. After internalized by tumor cell, (**I**) the complexes rapidly disassemble to release siRNA and free polyethylenimine (PEI) molecules in response to the acidic and reductive microenvironment, (**II**) efficiently escape from the endosome, facilitated simultaneously by cleaved PEI chains inducing membrane destabilization, the "proton sponge effect" of polyhistidine and polyethylenimine, as well as the relative small size of after disassembly, (**III**) achieve efficient gene silencing by cytosolic target mRNA cleavage. Adapted with permission from [179]. Copyright 2018 Elsevier.

Influenza-derived, histidine-rich H5WYG peptide that is capable of traversing intracellular barriers to deliver nucleic acids, is another well-known example that raised great interest. This pH sensitive peptide has been extensively applied to improve the gene delivery efficacy of different polymeric, peptidic, and lipid-based carriers [180–186].

Cationic LAH4 is another widely studied histidine-rich peptide where the protonation of the imidazole groups invokes chloride ion, as well as proton influx into the endosome, creating a hypertonic environment. Although the so-called "proton sponge effect", i.e., the osmotic influx of water triggering endosome lysis, has been recognized as the primary route of endosomal escape, other mechanisms also exist [187,188]. Changes in pH modulate the amphipathicity and membrane topology of LAH4 and derivatives: they are transmembrane at neutral pH, whereas under acidic conditions, LAH4 peptides align parallel to the phospholipid bilayer surface [189,190]. The interactions of the peptides with the bilayer interface eventually result in pore-formation [191] and membrane lysis [192], thereby modulating nucleic acid delivery. Inspired by the ability to enhance the efficiency of several gene delivery systems, researchers in the past extensively used LAH4 peptides as targeting moiety [187,193–198]. By now, there is a growing number of peptidic, as well as polymeric and lipidic carriers that utilize other histidine-rich moieties, such as $O_{10}H_6$ [199], $MS(O_{10}H_6)$ [200,201], histidine-rich Tat peptide [202–204], or His6 RPCs [205,206] to develop new promising gene delivery strategies. The possibility of intracellular delivery of nucleic acids in a nontoxic manner, which is a necessary prerequisite for gene therapy, has opened interesting perspectives in non-viral gene delivery. Nevertheless, there are many unanswered questions regarding the precise capacity or trafficking routes involved in favoring endosomal escape [172]. Hence, a comprehensive screen and quantification of

this step will provide further improvements for exploiting histidine-rich peptides in the field of gene therapy.

2.4. Peptides Assisting Delivery to Subcellular Organelles

A major focus in gene therapy is delivering nucleic acids to those intracellular compartments where they are therapeutically most effective. By escaping the endosome, siRNA and mRNA cargos arrive at their final destination, the cytosol, whereas DNA cargoes require translocation to the nucleus or to mitochondria. Intracellular targeting peptides serve a promising approach to specifically direct their cargo to the respective organelles and ensure membrane interactions that support delivery. Obviously, such peptides are particularly favorable candidates to be integrated into gene delivery systems [10,207]. The degree of translocation enhancement depends on the characteristics of both, delivery system and targeting peptide [208]. In the following sections, we address the mechanisms of intracellular nanoparticle trafficking and provide examples employing intracellular targeting peptides to ensure nuclear and mitochondrial targeting of nucleic acids.

2.4.1. Nuclear Localization Signals

Nucleocytoplasmic transport is major consideration for effective non-viral gene delivery [207,209]. Once inside the cell, most DNA must translocate into the nucleus where they can be either transcribed into the messenger RNA (mRNA) or interfere with transcription and RNA processing [210,211]. Nuclear localization signals (NLSs) are short peptide motifs rich in arginine, lysine, or proline that mediate nuclear translocation and when attached to foreign macromolecules or nanocarriers, deliver them to the nucleus [212]. For example, polymersomes, artificial vesicles resulting from self-assembly of amphiphilic copolymers, bypass the nuclear pore complexes (NPCs) that regulate transport into and out of the nucleus and deliver payloads directly into cell nuclei (Figure 6) [213]. Active transport of macromolecules to the nucleus is carried out by interactions of the NLS with importin receptors (karyopherins) and specific proteins of the NPC [214,215].

In view of the fact that the nuclear membrane is the main barrier restricting transgene expression of most non-viral carriers, gene therapy is the obvious field for the application of nuclear targeting peptides [216]. The significance of incorporating NLS peptides into non-viral delivery systems that can adequately favor the genetic materials release into the nucleus manifests itself by expanding case studies [207,217]. Hereby, positively charged NLS peptides either are attached to the negatively charged DNA via electrostatic interactions or are covalently coupled to the phosphate backbone of the DNA or to the condensing agent of the non-viral vector [216].

A frequently used NLS is derived from the large tumor antigen of Simian virus 40, SV40 (PKKKRKV). The positive charges of SV40 NLS peptide not only help in DNA condensation, but also mediate nuclear targeting [218]. Consistently, addition of the SV40 NLS peptide and its derivatives enhanced the transfection efficiency of many non-viral carriers [219–222].

Another interesting NLS is M9, a 38 amino acid peptide derived from heterogeneous ribonucleoprotein A1 (hnRNP A1) which is a major nuclear pre-mRNA binding protein. M9 is responsible for ferrying hnRNP A1 into the nucleus and also contains a nuclear export sequence (NES) [223]. Owing to its rather low positive charge, M9 is relatively poor at condensing DNA. On the other hand, it strongly interacts with its known receptor transportin 1 [224]. Therefore, utilizing the nuclear import effect of M9 in combination with positively charged biomaterials that condense the DNA offers great potential for gene therapy [142,225,226]. Furthermore, an NLS sequence derived from the HIV-1 viral protein (Vpr) promotes nuclear import through a karyopherin α–independent mechanism [227]. Examples for an enhanced transfection efficiency with Vpr-containing non-viral vectors are reviewed by Cartier and Reszka [217].

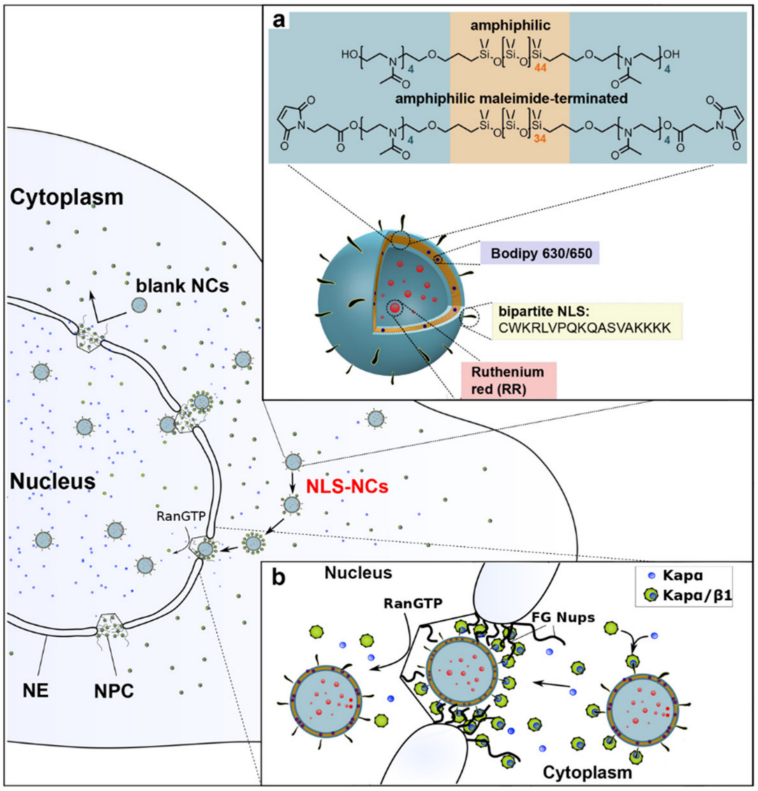

Figure 6. Organelle-specific targeting of polymersome NCs into the cell nucleus. (**a**) NLS-NCs self-assemble from amphiphilic PMOXA-PDMS-PMOXA triblock copolymers. Two model compounds are used to test for nuclear delivery: Ruthenium red (RR) that is encapsulated within the NLS-NC lumen, and Bodipy 630/650 that incorporates into its polymeric membrane. (**b**) The nuclear transport mechanism involves Kapα•Kapβ1 that (1) authenticates NLS-NCs for selective NPC transport, (2) binds to FG Nups, and (3) releases NLS-NCs into the nucleus upon binding RanGTP. Reprinted with permission from [213]. Copyright 2020 National Academy of Sciences.

Other examples of targeting sequences that facilitate nuclear transport of exogenous DNA include Xenopus protein nucleoplasmin [228,229], adenoviral peptide (Ad) [217,230], human T-cell leukaemia virus (HTLV) [224], Epstein–Barr virus nuclear antigen (EBNA)-1 [231]. By overcoming intracellular barriers, these peptides greatly expand the perspectives in non-viral gene delivery.

2.4.2. Mitochondrial Delivery

Mitochondria have their own genome whose mutations are associated with numerous disorders, such as cancer, diabetes, neurodegenerative diseases including Parkinson's disease, and more recently infectious and autoimmune diseases [225,226]. Given the link between mitochondrial dysfunction and disease, targeting of therapeutic interventions to these subcellular organelles is of vital importance [232,233]. Efficient mitochondrial gene therapy requires nanocarriers that, once inside the cell, target mitochondria and ferry nucleic acids across the outer (OMM), as well as inner mitochondrial membrane (IMM) [234]. The hydrophobicity and negative charge of MMs require that for efficient mitochondrial delivery, negatively charged DNA be shielded by carrier molecules, such as peptides that have amphiphilic and cationic properties.

Many natural and artificial short peptides and polypeptides have mitochondrial targeting ability [235,236]. Typically, these peptides comprise hydrophobic (phenylalanine, tyrosine, isoleucine) and positively charged (D-arginine, lysine) amino acids. The development of mitochondrion-targeted delivery strategies involving mitochondrial targeting sequences (MTSs), which are typically tens of amino acids in length, mostly takes advan-

tage of MM properties including the high negative potential (−160 to −180 mV) and the intrinsic protein import machinery. Although MTSs vary in length, they have in common an α-helical structure with an amphiphilic surface that mediates internalization by endogenous transmembrane transporters. However, because of their rather large size, low solubility and insufficient permeability across the plasma membrane, MTSs by themselves are not suitable for delivering exogenous nucleic acids.

In addition to MTSs that are recognized by translocators, several smaller peptides consisting of 4–16 cationic and hydrophobic residues efficiently target and permeate mitochondrial double membranes [235,237]. These mitochondria-penetrating peptides (MPPs), also known as mitochondrial CPPs (mtCPPs), typically appear to penetrate cellular membranes directly rather than by endocytosis [238]. Consequently, nanocarriers targeted by MPPs circumvent endosome/lysosome segregation, which also increases the chance of (gene) delivery to the mitochondria. In addition, MPPs appear to have marginal effects on mitochondrial membrane potential [239]

Considering that cell and mitochondrial membrane barriers have distinct compositions and properties, a single peptide will not be able to mediate the crossing of both. Here, combining CPP activity and mitochondrial targeting can act synergistically to optimize delivery of peptide-based DNA nanoparticles to mitochondria (Figure 7) [233,240]. For example, a library of fusion peptides with mitochondria targeting (mtCPP1) and cell-penetrating properties (Pepfect14, a stearylated CPP forming ASO nanocomplexes with splice-correction activity in cells [241]) that self-assembled with antisense oligonucleotides (ASO) into complexes was successful in knocking down mitochondrial mRNA [242]. A combinatorial approach to develop mitochondrial gene expression was also pursued by incorporating an MTS into WRAP peptides (short tryptophan/arginine rich peptides; [243]) which formed nanocomplexes with plasmid DNA encoding the mitochondrial ND1 gene that were taken up by cells and targeted to mitochondria [244]. Systematic analysis of CPPs and MTSs revealed that while both types of peptides were rich in Ala and Arg, the latter included Leu, suggesting a role for Leu in targeting to mitochondria [245].

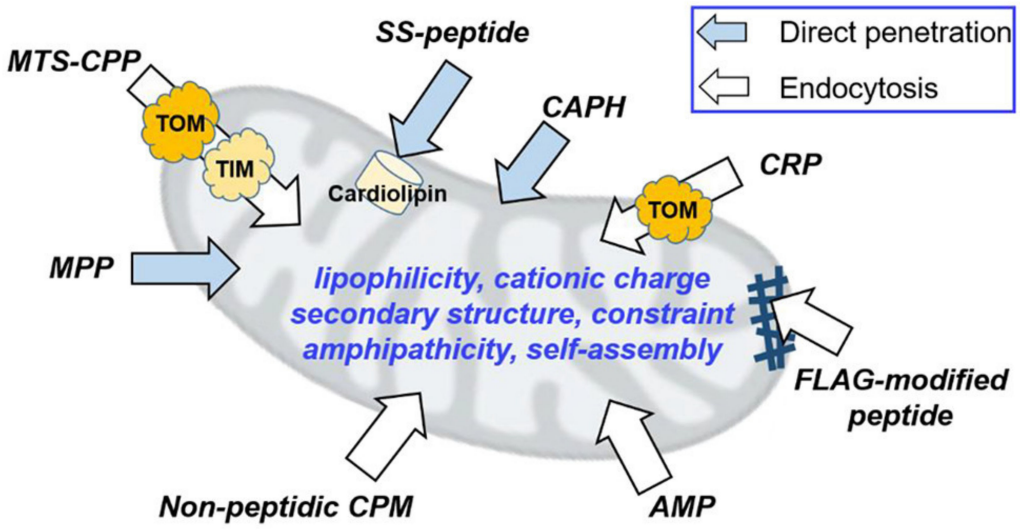

Figure 7. Mitochondrion-targeting peptides and peptidomimetics based on their structural classes and reported applications. Abbreviations: MTSCPP, MTS with cell-penetrating peptides; MPP, mitochondrion-penetrating peptides; SS-peptides, Szeto−Schiller peptides; CAPH, cationic amphiphilic polyproline helix; CRP, cysteine-rich peptides; FLAG-modified peptide, FLAG tag-based peptide that self-assembled into a nanofiber; AMP, peptides derived from antimicrobial peptides; CPM, nonpeptidic cell-penetrating motif; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; IMS, intermembrane space. Reprinted with modification from [233]. Copyright 2020 American Chemical Society.

3. Peptide-Related Nano-Assemblies for Nucleic Acid Delivery

Peptides have great potential as self-assembly building blocks on account of primary and secondary structure variability. Depending on the design, they form various supramolecular assemblies, such as vesicles [246], micelles [247], nanotubes [248], nanofibers [249], or nanoribbons [250]. Choosing corresponding peptide building blocks allows for tuning size and shape of the nano-assembly to obtain improved nanocarrier properties including cargo loading and delivery. Moreover, the weak interactions involved in peptide self-assembly are sensitive to environmental conditions, enabling nano-assemblies to exhibit specific functionalities in response to different external stimuli, such as temperature, pH, redox state, enzymes, or even light. We first focus on examples of supramolecular assemblies where peptides represent the predominant building block of the nanocarriers or are integrated into the nanocarriers to improve their targeting and gene delivery properties, and then discuss examples with stimuli-responsiveness.

Prominent structures that serve as nucleic acid carriers are micelles entrapping DNA during self-assembly, also called "micelleplexes" [251]. If purely peptidic, micelleplexes possess minimal cytotoxicity [20]. However, often peptides are used as a targeting or uptake-facilitating moieties associated with nanocarriers made out of different, less biocompatible materials. A micelle forming polymer-peptide conjugate used as an siRNA carrier has recently been reported as an effective tool in anti-metastasis cancer therapies (Figure 8) [252]. Methoxy-polyethylene glycol combined polycaprolactone conjugated with a cytoplasm-responsive peptide CH2R4H2C (MPEG-PCL-CH2R4H2C) was used to entrap anti-RelA siRNA (siRelA). RelA is a subunit of NF-κB involved in metastasis, especially cancer cell migration and invasion. The MPEG-PCL part of the conjugate was expected to improve blood retention and tumor accumulation and to facilitate micelle formation. Consistent with this notion, siRelA/MPEG-PCL-CH2R4H2C micelleplexes successfully delivered siRNA into cancer cells in a lung metastasis mouse model, causing inhibition of RelA accompanied by significant suppression of metastasis.

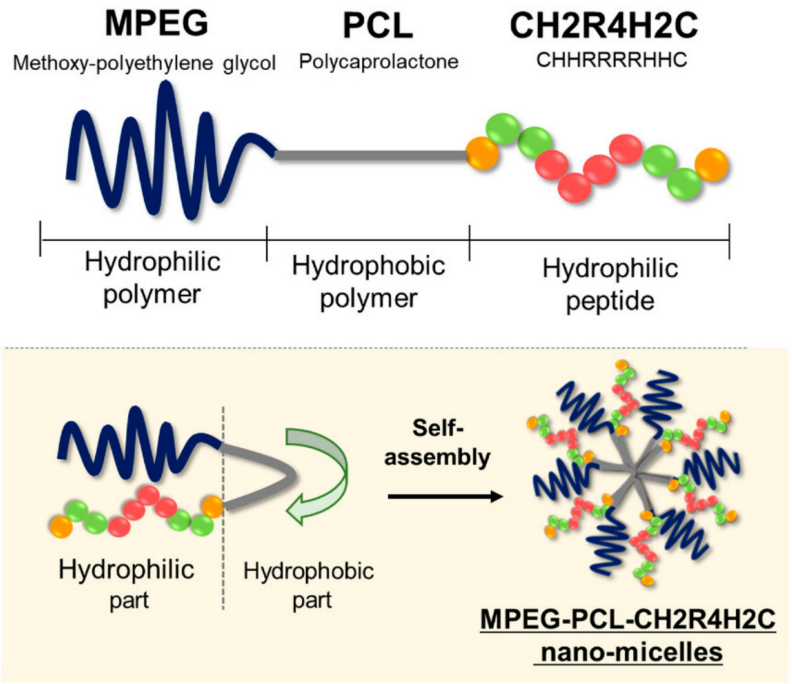

Figure 8. The structure of MPEG-PCL-CH2R4H2C and nano-micelle formation. Reprinted with permission from [252]. Copyright 2020 Multidisciplinary Digital Publishing Institute (MDPI).

More recently, peptide-assisted polymeric micelles were used for inhibiting the mitotic cycle of prostate cancer cells by siRNA delivery in cell lines and in tumor-burdened nude

mice [253]. The hydrophilic segments of acetal-polyethylene oxide-*b*-polycaprolactone (A-PEO-PCL) copolymers were chemically modified with a TAT peptide and a ligand for prostate-specific membrane antigen (DCL) to enhance targeting and cell penetration of self-assembled micelles loaded with siRNA and docetaxel (anti-cancer drug).

Other small molecules, for example a palmitoyl chain conjugated to the N-terminus of GGGAAAKRK [254], proved useful in promoting self-assembly of peptides to distinct nanocarriers with a hydrophobic core. Accordingly, surfactant-like palmitoyl-GGGAAAKRK formed peptide nanofibers (PNFs) in the presence of siRNA specific for the down-regulation of BCL2 protein. Human SH-SY5Y cells showed significant uptake of PNF:siBCL2 constructs in vitro and silencing of *BCL2* in specific loci of rat brains demonstrated effective delivery of siRNA. In another example, a branched amphiphilic peptide comprising oligolysine segments with DNA binding properties formed different structures depending on the peptide/DNA ratio: at high peptide/DNA ratio, it coated the DNA surface forming nanofibers and at low peptide/DNA ratio, it condensed the DNA into nanometer-sized compacted structures [255]. Using pDNA encoding green fluorescent protein (GFP) as cargo, the peptide nanocarrier demonstrated higher transfection efficiency in HeLa cells compared to Lipofectin (commercial transfection agent) when the total number of transfectants alive was considered.

Another type of versatile and reproducible supramolecular nanocarrier with well-defined structure and composition are dendrimers [256]. Many types of dendrimers including peptide dendrimers (PPI; [257]), poly(L-lysine) dendrimers, and polyamidoamine (PAMAM) dendrimers that display electrostatic interactions with nucleic acids and protect the cargo from degradation, are particularly suited for gene delivery. In addition, dendrimers lend themselves to surface conjugation of peptide moieties that enhance gene delivery. Conjugation of TAT (HIV transactivator of transcription) peptide to PAMAM dendrimer formed nanometer-sized (105 nm–115 nm) 'dendriplexes' with GFP pDNA [258] that displayed an increased transfection efficiency in Vero cells compared to PAMAM without TAT.

Stimuli-Responsive Gene Delivery Systems

Peptide-based assemblies are particularly attractive for developing stimuli-responsive therapeutic nanocarriers for several reasons: they are biocompatible, readily degraded and then removed from the organism, but most importantly, highly sensitive to environmental conditions. Small changes in external factors, such as temperature or pH, can induce transformation of secondary structures (α-helices, β-sheets, and β-turns), thereby affecting the morphology and concomitantly the function and bio-activity of the polypeptides [259]. Moreover, control over cargo release is a highly sought-after feature in gene delivery systems and stimuli-responsive nanocarriers can deliver nucleic acids more efficiently by reducing unspecific release. To date, peptide vectors forming complexes with DNA by electrostatic interactions still make up the majority of peptide-based nanocarriers. As stimuli-responsiveness can be readily obtained by modifying the peptide sequence, these nanoparticles (peptiplexes) represent the main targets for a control of cargo release by external stimuli. However, nano-assemblies, based on their modularity offer not only increased DNA loading capacity but are also more susceptible towards environmental stimuli.

The pH-responsive peptides have been intensively investigated in delivery and diagnostic systems because pH variations are typical for many biological systems, intracellular compartments (lysosomes and endosomes), specific organs (gastrointestinal tract and vagina), and pathological conditions [260,261]. Particularly, the microenvironment of many tumor tissues has a lower pH (<6.5) compared to normal tissues (pH 7.4). Thus, polypeptides that change conformation in a pH-dependent fashion can find application in selective binding to cancer sites which can be exploited for tumor diagnosis and treatment. Nanocarriers taken up by endocytosis encounter acidification in the endosome, which is exploited by pH-responsive peptides to increase endosomal escape. For example, (Fmoc)2KH7-TAT,

an amphiphilic, pH-responsive chimeric peptide [262], complexed with pGL-3 reporter plasmid mediated transfection of 293T and HeLa cells by promoting endosomal escape via protonation of KH residues. Moreover, co-delivery of p53 plasmid and doxorubicin using (Fmoc)2KH7-TAT self-assembled micelleplexes inhibited cell growth in vitro and tumor growth in vivo.

Nanocarriers with peptide-mediated redox-sensitivity have emerged as a fascinating type of biomedical material with potential for triggered gene and drug delivery inside cells. Sensitivity of peptides to the redox state is provided by disulphide bonds, diselenide bonds, succinimide-thioether linkage or by redox sensitive groups, such as "trimethyl-locked" benzoquinone [263]. In a reducing environment, redox-responsive nanocarriers undergo a change in conformation and release their cargo. A major advantage of redox-responsive nanocarriers is their stability in normal tissues which avoids cytotoxicity caused by the unwanted release of therapeutic cargo. Tumor tissues show 4-fold higher glutathione levels compared to healthy tissue and thus triggers redox-responsive cargo release [264].

A "smart", redox-sensitive peptide designed to trigger the assembly of gadolinium nanoparticles inside cells, was successfully applied in magnetic resonance imaging of tumors in a xenograft mouse model [265]. Acetyl-RVRR-C(StBu)-K(Gd-DOTA)-CBT contains an RVRR sequence which mediates cell membrane translocation but is also a cleavage site for intracellular furin, typically upregulated in many tumors, and a disulphided Cys motif. After entering the cell, the disulfide bond is reduced by intracellular glutathione (GSH) and subsequently, the RVRR motif is cleaved by furin in situ. The cleavage product quickly condenses to amphiphilic dimers that self-assemble via π-π stacking into Gd-containing nanoparticles.

Peptide structures and the weak interactions contributing to self-assembly of peptide-based nanocarriers are inherently sensitive to temperature [266,267]. An interesting example of a thermo-responsive, purely peptidic DNA nanocarrier are multi-compartment micellar nanoparticles (MCM-NPs) assembled from (HR)3gT peptide (Figure 9) [20].

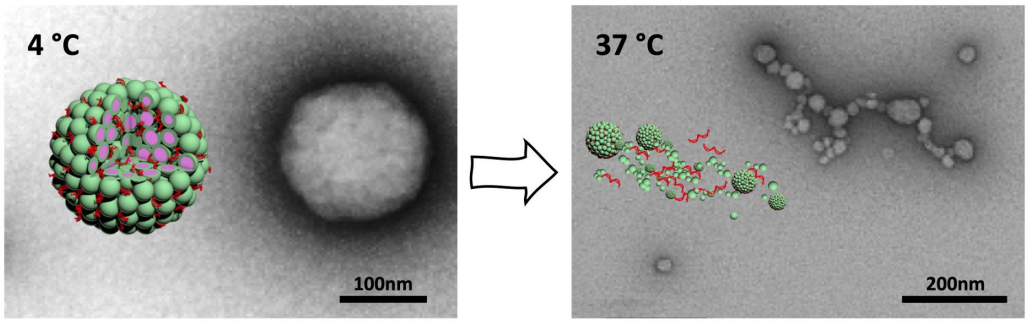

Figure 9. Schematic representation and TEM micrograph of the self-assembled (HR)3gT multi-compartment micellar nano-assembly (MCM) at 4 °C (*left*) and temperature-induced disassembly of MCMs into disperse or clustered smaller MCMs and individual micelles at 37 °C (*right*). Modified from [20] with permission from the Royal Society of Chemistry.

Although the multicompartment micellar structure of NPs was stable at 4 °C, increasing the temperature to 37 °C triggered structural changes that led to the disassembly into smaller MCMs and individual micelles after several hours. On account of the high cellular uptake efficiency and thermo-responsive disassembly at physiological temperature, MCM-NPs are a promising DNA delivery vehicle with great potential for application in vivo. Similar multicompartment micellar NPs assembled from H_3SSgT peptide bearing a disulfide functional group between hydrophilic and hydrophobic domain, were developed for redox-responsive codelivery of oligonucleotides and drugs [268]. The disulfide bond conferred responsiveness to physiological concentrations of reducing agent upon NPs, resulting in release of the incorporated cargo. The advantage of a supramolecular multicompartment structure over individual micelles lies in the increased capacity for

oligonucleotide condensation [269]. Together with the ability to entrap various hydrophobic cargos, this makes MCM-NPs well-suited for biomedical applications.

Light has received much attention as an external stimulus, as it provides spatiotemporal control that can be triggered remotely. By crosslinking peptides with specific light-absorbing molecules it is possible to obtain photo-responsive conjugates that allow for light-stimulated assembly of nanostructures or light-induced release of cargo molecules. Such light-sensitive conjugates of peptides and photosensitizers can serve as light-controllable phototherapeutic agents [270].

Photo-crosslinking by UV (254 nm) of poly(ethylene glycol)-*b*-poly(l-glutamic acid) diblock copolymer was shown to convert the core of self-assembled core-shell micellar structures to nanogels that, depending on the composition of the copolymer, could release drug payload in a pH-dependent manner [271]. These results indicated the potential of nanogels fabricated by photo-crosslinking of polypeptide micelles as intelligent delivery systems.

Micelles with a photocleavable poly(S-(o-nitrobenzyl)-l-cysteine) (PNBC) core surrounded by a hydrophilic poly(ethylene glycol) (PEO) corona were also obtained by self-assembly of PNBC-b-PEO amphiphilic block copolymer [272]. UV irradiation (365 nm) of these micelles gradually removed nitrobenzyl groups from PNBC-b-PEO resulting in a shrinkage of the micelles. If micelles were prepared in the presence of doxorubicin, a photo-triggered release of the drug was observed in vitro. Since self-assembly is achieved in aqueous solution, photocleavable polypeptide-based block copolymers lend themselves to developing photoresponsive nanomedicines for anticancer therapy.

4. Combinatorial Approach for Advanced Nucleic Acid Delivery

In view of gene therapy, endowing nanocarries with targeting features and stimuli-responsiveness that provides site-specific, triggerable control over cargo release could optimize delivery efficacy, and, at the same time, minimize adverse effects.

For example, folate-receptor targeting, acid-sensitive polymeric micelles (F-ASPM) have been successfully applied to deliver siRNA to breast cancer cells (Figure 10) [273]. In this approach, poly (L-histidine)-polythelene-glycol (PEG-PHIS) and folate-conjugated PEG-PHIS amphiphilic block copolymers in the presence of CPP-coupled siRNA self-assembled into micelles where folate functioned as targeting ligand and histidine residues provided pH-responsiveness. As PEG-PHIS block copolymers have a pKb of 6.5–7.0, micelles formed by this copolymer dissociate at pH 6.5–7.0 which renders them suitable for constructing drug/DNA delivery systems that are sensitive to the extracellular tumor environment. In addition, c-myc silencing siRNA conjugation to a CPP was obtained by reduction-sensitive disulfide bonding which turned the resulting micelles into a dual responsive nanocarrier able to target tumor cells where release and delivery of siRNA are promoted by the respective peptide sequences.

The combination of targeting and stimuli-responsiveness is also provided by ROSE, a redox-sensitive, oligopeptide-guided, self-assembling, and efficiency-enhanced carrier system [274]. In ROSE, adamantyl-PEG chains with and without disulfide bonded SP94 targeting oligopeptide, mixed with hydroxypropyl-β-cyclodextrin formed supramolecular complexes condensing tumor-suppressor microRNA-34a (miR-34a). Oligopeptide-guided specificity for hepatocarcinoma cells and release of miRNA following disulfide cleavage in the reducing environment significantly improved the tumor-suppressing effect of ROSE/miR-34a over conventional gene delivery strategies.

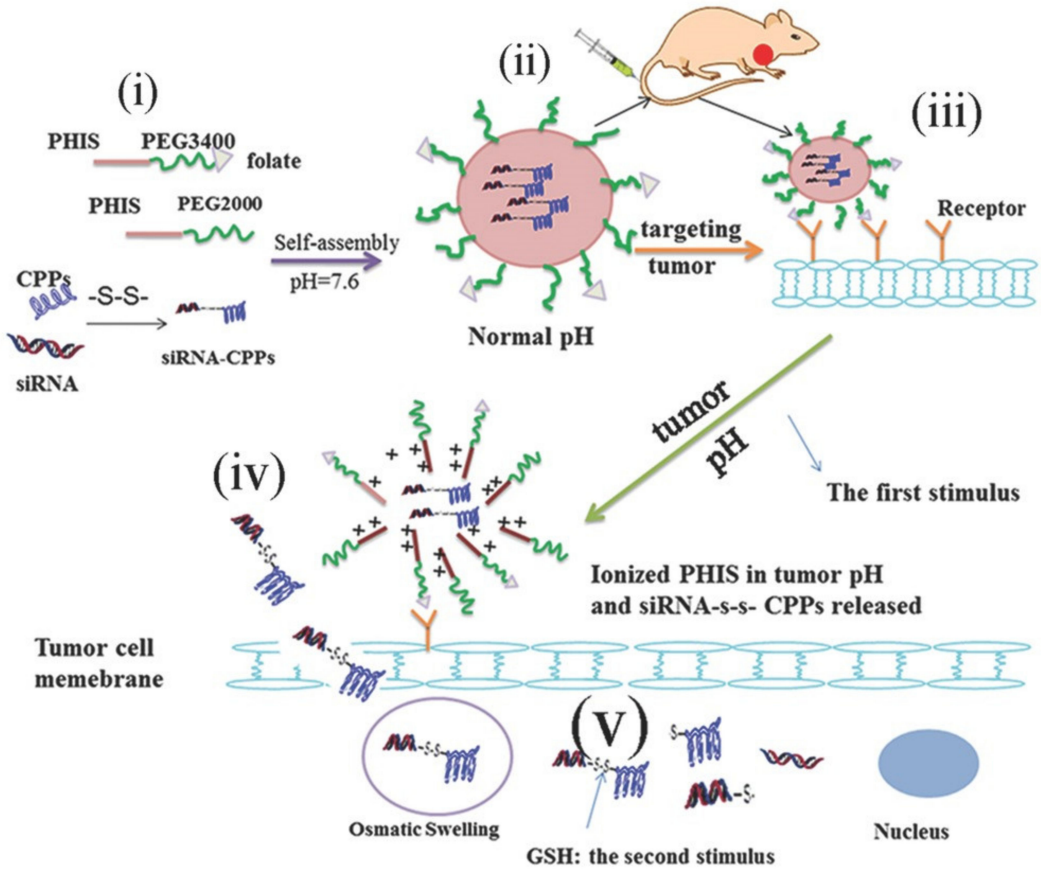

Figure 10. Diagram of F-ASPM formation and the mechanism of siRNA delivery into cancer cells. (**i**) synthesis of PEG-PHIS and F-PEG-PHIS. (**ii**) self-assembly of amphiphilic block copolymers in the presence of siRNA-CPP into acid-sensitive nanocarriers with active targeting ability (F-ASPM). (**iii**) binding of F-ASPM to cancer cells by folate receptor targeting. (**iv**) pH-stimulated release of siRNA-CPPs from F-ASPM. (**v**) GSH-mediated cleavage of disulfide bond of siRNA-CPPs leading to release free siRNA into cytosol. Reprinted with permission from [273]. Copyright 2016 John Wiley and Sons.

5. Conclusions

Since the introduction of peptides as potential delivery system for a variety of therapeutic cargos, extensive research has focused on their application in gene therapy. To be suitably tailored for gene therapy, peptide-based nanocarriers must comply with issues of targeting, cellular uptake, and intracellular trafficking, all of which involve biological membranes and how they can be overcome. A combinatorial approach, e.g., designer peptides composed of cationic cell-penetrating and hydrophobic endosomal escape domains in combination with a gene carrier peptide composed of targeting and cationic DNA-binding domains affording triggered, site-specific (cytosol, nucleus, mitochondria) release of nucleic acids, may offer some improvement of efficacy. Other properties, including, but not limited to, low cytotoxicity, target specificity, biodegradability, and cost and time efficiency of synthesis greatly contribute to the potential of peptides in nanomedicine. Nevertheless, to broadly realize bench to bedside translation of peptide-related gene delivery systems, innovative technologies need to be pursued to achieve peptide-based nanocarriers that more specifically and efficiently deliver nucleic acids or nucleic acid modifying systems to the desired sites. In many cases, such nanocarriers would further benefit from either sustained or triggered delivery options.

Advances in peptide development have made peptide-assisted gene delivery more efficient in vitro and, in some instances, in small animal models [275]. For example, cell and tissue selectivity could be greatly enhanced in the newest generation of CPPs [276]. Other advances which allow for improved performance with regard to targeting and

delivery of nucleic acids include adapting peptide sequences to facilitate escape or release from intracellular vesicles or respond to environmental stimuli for a controlled release of cargo, and the development of composite, multivalent peptide-based, or peptide-coupled structures.

Intriguingly, while revolutionary and versatile peptide tools have inspired a great deal of hope regarding the treatment of genetic diseases, peptide nanocarriers are awaiting clinical translation. For example, none of the nanocarriers associated with CPPs have so far been approved for clinical studies. Evidently, besides overcoming membrane barriers, a string of challenges remain that need to be tackled for peptide nanocarriers to make a breakthrough in clinical application whereas lipid-based formulations, for all their drawbacks [277], are being used in the field of gene therapy and as a delivery vehicle in mRNA-based vaccines [278]. Short circulation half-lives, inadequate biodistribution, and poor chemical and physical serum stability, especially susceptibility to proteolytic degradation associated with off-target nucleic acid release, hamper clinical translation of peptide-based nanocarriers. Refining their preparation with regard to gene loading efficiency and product homogeneity would be a possible improvement. However, reaching the final target with high selectivity and adequate accumulation at the target site remains a major issue for in vivo applications. Here, peptide modifications (unnatural amino acids, cyclization) and conjugate molecules (PEGylation, hydrocarbon chains) that prolong the circulation time and enhance the structural stability of nanocarriers in the serum come to mind [279,280]. Peptidomimetics, often based on natural peptide sequences, that exhibit improved proteolytic stability or even new folds and morphologies designed to enhance bio availability, improve transport through the blood–brain barrier, or reduce the rate of clearance, are emerging. However, their modifications bear the risk of reducing potency or even introducing toxicity, for example D-amino acids [281]. Another alternative to clear abovementioned hurdles is developing advanced multifunctional carriers comprising various agents, each of which can overcome the barrier through distinct dictated functions. Undoubtedly, peptides offer the largest potential when it comes to nucleic acid condensation, targeting, endosomal escape, and subcellular localization as a part of multifunctional advanced delivery systems. But again, the combination of functionalities bears the danger of affecting the individual functions. Thus, extensive research on the development of stimuli-responsive purely peptidic systems with suitable physicochemical properties for nucleic acid delivery is being pursued at many levels.

A better understanding of the mechanisms by which peptide-based delivery systems use to overcome membrane but also other biological barriers, together with advancements in the synthesis of innovative materials tailored to environmental conditions and extensive in vivo studies herald a bright future for peptide-based delivery systems in gene therapy and in nanomedicine in general.

Funding: Swiss Nanoscience Institute (SNI), NCCR Molecular Systems Engineering, University of Basel, Switzerland.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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