CELL-PENETRATING PEPTIDES: A DELIVERY SYSTEM FOR OLIGONUCLEOTIDES

Oligonucleotides are very promising and versatile therapeutics for rare and genetic diseases. They act on the RNA level through different molecular pathways that we already have described in a precedent article. Because of their mechanism of action, this class of therapeutics has huge possibilities to treat diseases that were incurable before. The use of oligonucleotide therapeutics is well tolerated by the body, selective to their target, and with reduced secondary effects. Despites their huge potential and attractiveness as a treatment, there is a major drawback when dealing with oligonucleotides as drugs: their poor bioavailability and cellular uptake. These aspects are the main limitations for their application as therapeutics. To address these drawbacks, it seems of utmost importance to develop delivery systems that enable oligonucleotides to reach their targets. In a previous article on oligonucleotide conjugates, we have described the use of the trimeric GalNAc (N-acetylgalactosamine) molecule as a carrier of oligonucleotides to specific cells, the hepatocytes. Here, we discuss a delivery system that have been in vogue since almost 40 years, the cell-penetrating peptides (CPP). The first use of CPP, a poly L-lysine, has been described for the delivery of an anticancer drug *in vitro* and *in vivo* of mouse model[1]. It has opened the way for generation of CPP-drug conjugates and has potential as well for oligonucleotides[2].

WHAT ARE CELL-PENETRATING PEPTIDES?

CPP are short peptides that are able to translocate small so-called cargo molecule across cell membranes[3]. They usually consist of no more than 30 amino acids and are cationic and/or amphipathic, rich in arginine and lysine amino acids. A variety of different CPPs exists, reaching from natural translocating proteins as the earliest developed CPP to newly designed sequences, the latter based on computer prediction[4]. The mode of action is as follows: First, the CPP – conjugated to its cargo molecule – is internalized through endocytosis. It is then trafficking in the endosomal compartment where it is entrapped. Finally, the CPP should be able to escape the endosomal compartment to deliver the cargo molecule to the target. The ability of the CPP to escape this compartment is key for its bioavailability and bioactivity properties. Important criteria for a CPP system delivery are to enhance the cellular uptake, endosomal escape, cell membrane receptor binding, and nuclear localization.

HOW TO CONJUGATE CELL-PENETRATING PEPTIDES TO OLIGONUCLEOTIDES?

There are two main vectorization strategies for CPP: the covalent conjugation to its cargo molecule and the nanoparticle formation-based approach.
For a covalent linkage, thiol-maleimide coupling has been the method of choice. It is a widely used reaction in peptide chemistry and approved by the regularity authorities for several antibody-drugs conjugates[5]. At Bachem we have extensively studied this reaction and the side-reactions that occur during the coupling onto a peptide. You can read our blog article and check out our webinar replay to learn more on the thiol-maleimide coupling.

Please embed this youtube video here, if possible: https://youtu.be/9YDCj-bDrAo

However, this method has a major challenge when it comes to purify the CPP-oligonucleotide conjugate. Since CPP are positively charged and oligonucleotides are negatively charged, they are prone to aggregation leading to precipitation of the conjugate. Recently, a methodology to couple a CPP to an antisense oligonucleotide efficiently via the thiol-maleimide reaction has been published[6]. The researchers demonstrated that highly cationic CPPs can be conjugated to 2'-O-(2-Methoxyethyl) phosphothioate oligonucleotides, and that they can be isolated in good yields.
The nanoparticle formation-based approach takes place on basis of electrostatic and hydrophobic interactions between the CPP and the cargo. Typically, this strategy is used for the delivery of small interfering RNA (siRNA). One big advantage is that it can be applied for larger oligonucleotides. However, the particles formed are unstable in physiological fluids if the CPP are not chemically modified. To tackle this challenge, chemical modifications of the CPP are essential.

CELL-PENETRATING PEPTIDES CONJUGATES HAVE BEEN USED TO DELIVER OLIGONUCLEOTIDES

Although the delivery of oligonucleotides by CPP represents a very attractive approach, only a handful of examples have been reported in the literature for *in vivo* application. Some recent examples are described in the table below.

CPP	Oligonucleotide	Conjugation strategy
WRAP5	siRNA	Nanoparticles (7)
PepFect6	Anti-miRNA	Nanoparticles (8)
cRGD	siRNA	Covalent (thiol-maleimide) (11)
GLP1R	ASO	Covalent (disulfide bridge) (12)

In a recent publication, scientists from university of Bordeaux reported about a CPP-based nanoparticles approach, developed to deliver siRNA into cancer cells of solid tumors. They could show the *in vivo* delivery efficiency of a new peptide WRAP5. The peptide is able to wrap the siRNA and then forms nanoparticles[11].
In a second work, CPP-based nanoparticles are applied as a vector for an anti-miRNA. Anti-miRNA (AMO) are synthetically designed oligonucleotides. They are used to neutralize the so-called microRNA (miRNA), which are short complementary sequences to messenger RNA (mRNA) and involved in the suppression of the translation process. CPP-AMO nanoparticles are used for tumor imaging[12]. The major component is a PepFect6 peptide encapsulating the AMO labeled by a radiotracer used for imaging the miRNA-21 expression in lung adenocarcinoma xenografts. PepFect6 improves the cellular delivery of the conjugated oligonucleotides and has shown promising results for *in vivo* imaging of miRNA.
The cRGD (cyclic(arginine-glycine-aspartic)) peptide is a common CPP used to target αvβ3 integrin receptors. Integrin αvβ3 is involved in angiogenesis and tumor metastasis and is up-regulated in tumor cells of many cancer types[13]. Some examples of cRGD-oligonucleotide conjugates have been reported in the literature to enhance cellular uptake, subcellular distribution, and pharmacological effects of the cargo oligonucleotide[14]. In the following example of a CPP-oligonucleotide conjugate, a derivative of cRGD peptide, cyclo(Arg-Gly-Asp-d-Phe-Lys[PEG-MAL]) (MAL: maleimide), has been covalently conjugated to a siRNA[15]. In *in vitro* experiments, the conjugate has demonstrated to specifically enter αvβ3 positive human cells and to silence the targeted genes. Following these promising results, the cRGD-siRNA conjugate has been injected in tumor-bearing mice and has shown very positive results. In addition to be well tolerated and well distributed in the tumor tissues, the injection has induced a down-regulation of corresponding messenger RNA and protein resulting

in a significant reduction of the tumor volume, down to 90%. cRGD-oligonucleotide conjugates have potential as anti-tumor therapeutics.
A last example highlights glucagon-like peptide-1 receptor (GLP1R) as a vector for delivering antisense oligonucleotides (ASOs). In the literature, GLP1R is described to be unsuitable for selective drug delivery mainly due to its low abundance and limited ability to internalize a large amount of drug conjugate. In a publication from 2018, researchers have developed a new approach that uses GLP1R as an internalization inducer to deliver an ASO to the pancreatic β-cells[16]. The covalent conjugation via a disulfide bridge between GLP1R and the ASO has enhanced the selective cellular uptake of the ASO in the targeted cells. The peptide conjugation also improves the potency of the ASO in a GLP1R-dependent manner and induces the gene expression silencing that leads to a reduction in protein levels. Furthermore, it has been demonstrated that GLP1R is not only able to deliver the ASO to the pancreatic β-cells but also into the pancreatic islets in the liver with an enhanced uptake. This work opens the door to a new treatment options for diseases caused by an aberrant gene expression in pancreatic β-cells, like diabetes.

AT THE CROSSROADS BETWEEN PEPTIDES AND OLIGONUCLEOTIDES

CPP represent a great opportunity to overcome the delivery issue and bad bio-distribution of oligonucleotides. They enable the specific delivery of their cargo molecule to their target cells and tissues. Either linked covalently or complexed into nanoparticles with their cargo, CPP provide a huge mobility advantage and a critical access to the most challenging tissues, such as muscle, bone marrow and barriers, such as the blood-brain barrier. CPP-oligo conjugates combine two core competencies of Bachem, peptide and oligonucleotide synthesis. Since 50 years, we provide high quality peptides for various applications and have collected a track record of about 80 Drug Master Files (DMFs) filed. Recently, we expanded our heritage of peptide expertise to oligonucleotides. Similar to peptides, oligonucleotide therapeutics require expert knowledge in solid-phase synthesis and protecting group chemistry. Downstream processing typically includes the same steps such as chromatography, ultra- and diafiltration techniques, precipitation and lyophilization. We have seen the demand in the oligonucleotides getting higher and higher to provide new drugs to cure rare and genetic diseases. Thus, we have built our capabilities for large scale production of oligonucleotides compliant with GMP requirements. With our strong innovation program, we offer our customers the solutions they need to supply large volume of oligonucleotide drugs on a sustainable way and can tackle the challenges of pharma and biotech companies to transform patient’s lives.

ABOUT BACHEM

Bachem is a leading, innovation-driven company specializing in the development and manufacture of peptides and oligonucleotides.
With over 50 years of experience and expertise Bachem provides products for research, clinical development and commercial application to pharmaceutical and biotechnology companies worldwide and offers a comprehensive range of services.
Bachem operates internationally with headquarters in Switzerland and locations in Europe, the US and Asia. The company is listed on the SIX Swiss Exchange.

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