

POSTERS SUBMITTED TO DATE

1. New Palmitoylated Analogs of Prolactin-Releasing Peptide as Potential Anti-Obesity Agents

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Abstract:

Anorexigenic neuropeptides produced and acting in the brain have the potential to decrease food intake and ameliorate obesity but are ineffective after peripheral application. We have designed lipidized analogs of prolactin-releasing peptide (PrRP) which is involved in energy balance regulation as demonstrated by obesity phenotypes of both PrRP- and PrRP-receptor-knockout mice.

Our analogs of prolactin-releasing peptide with palmitic acid attached through glutamic acid or short chain of polyethylene glycol at Lys¹¹ showed binding affinity and signaling in cells with PrRP receptor similar to natural PrRP. Peripheral administration of these analogs to free fed mice and rats induced strong and long-lasting anorexigenic effect and neuronal activation in the brain areas involved in food intake regulation. Moreover, these novel analogs revealed higher bioavailability compared to PrRP analogs palmitoylated at N-terminus without any linker, probably due to higher solubility and better absorption after subcutaneous administration in mice and rats.

Our data suggest that lipidization of PrRP enhances its stability, offer possibility to deliver the analogs peripherally and enables the peptide to cross blood-brain barrier. Strong anorexigenic and body-weight-reducing effects make lipidized PrRP analogs attractive candidates for anti-obesity treatment. This study was supported by GACR 15-08679S, TACR TE01020028, RVO:61388963 and RVO:67985823.

2. Ion-Pairing Systems for Reversed-Phase Chromatography of Oligonucleotides

Authors: Robert Birdsall, Sean M. McCarthy, David Lascoux and Martin Gilar

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Abstract:

Synthetic oligonucleotides are produced using highly efficient solid phase processes which have been optimized to produce very high yields, often above 99% per synthetic step. Following synthesis, it is common to assess purity of the final product. Commonly, this is accomplished using chromatographic methods, such as ion exchange (IEX) or ion pairing reversed phase chromatography (IP-RP). Recently, IP-RP methods have become more popular due to their compatibility with MS detection and equivalent or better resolution to IEX in a fraction of the time. In this study we will discuss a systematic comparison of the resolving power and MS compatibility of ion-pairing systems for oligonucleotide analysis. Our study focuses on the comparison of a variety of alkyl amines of varying

hydrophobicity. To evaluate each amines suitability for IP-RP separations, we compared separations at different concentrations by determining their peak capacities for a homopolymer oligonucleotide separation. We selected the higher performing amines and performed separations of heteropolymers to assess each systems ability to separate by charge while minimizing hydrophobic contribution. Our results show that both triethylamine/hexafluoroisopropanol (TEA/HFIP) and hexylammonium acetate (HAA) provide the greatest peak capacities and separations largely driven by charge. We found that HFIP is best suited for MS analysis.

3. The Versatility of Recombinant Human Albumin in the Formulation and Delivery of Peptide Therapeutics

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Abstract:

The growing field of peptide therapeutics gives promise for improved treatments against many diseases. However many of the peptides found to be efficacious face challenges in formulation and use due to hydrophobicity or fast systemic clearance due to their small size.

We present here the use of recombinant human albumin (rAlb) as a means to address these challenges. We find rAlb to be superior to human-plasma-sourced albumin in purity and homogeneity, making rAlb the preferred choice when developing formulations or half-life extension based on human albumin.

Human albumin is an obvious candidate for serum half-life extension; it is non-immunogenic and has a natural serum half-life of 19 days. We have designed albumin variants that have increased binding affinity for the neonatal Fc receptor (FcRn), thereby improving their recycling by the FcRn and increasing their serum half-life even further. Peptides that are in need of increased serum half-life can either be chemical conjugated to the free thiol of rAlb, or genetically fused to the rAlb. Examples of both strategies are shown, illustrating the robustness of the FcRn interaction for the albumin-drug conjugates.

Human albumin has many biophysical properties. It binds a wide range of molecules, typically hydrophobic, negatively charged ones. Albumin has also been shown to cover surfaces, both hydrophobic and hydrophilic, in a near-to-one protein layer. These properties are demonstrated in stabilizing the otherwise highly fibrillating Teduglutide and in preventing adsorption during handling of Glucagon.

4. Development of Peptide-siRNA-Prodrugs

Author:

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Abstract:

In nucleotide development several hurdles including effective delivery and cellular targeting have to be overcome to induce therapeutic phenotypes. In oncology drug development, target selection is a

major challenge since cancer cells are flexible regarding gene expression, signalling and tolerate substantial levels of genetic instability.

We developed siRNAs each targeting multiple genes important for cellular survival and having the potential to be used as cancer toxins.

To control RNAi-based toxic effects, a prodrug-targeting approach was developed to protect liver, spleen and kidney. Peptides bound to the 5' antisense strand inhibit biological activity; peptides are targets of specific cytoplasmatic proteases. Since protease expression differs between cellular subtypes and cancer cells, peptide cleavage and siRNA activation occurs selectively within desired target cells.

Biodistribution, tolerability and efficacy were investigated in mammary cancer mouse xenograft experiments. Intravenous dosing of 3mg/kg LNP formulated candidates showed excellent tolerability. Highest amounts were found in liver, lung and kidney. Drug candidates were also detected in tumor. No RNAi and toxic phenotype was detected in liver, whereas gene expression was reduced in cancer tissue, proliferation was reduced; apoptosis was induced. In conclusion peptide-RNA-prodrugs have a high therapeutic potential for effective tumor treatment.

5. 1D diffusion NMR in development of co-formulations of pharmaceutical peptides

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Abstract:

As metabolic conditions such as obesity, type II diabetes and related co-morbidities are on the rise, the world is looking for new therapeutics. Since many metabolically active peptides/proteins act in unison in the body, pharmaceutical companies are beginning to look into combining them in co-formulations to achieve similar synergistic effects from a single injection. Recent examples of successful combinations can be seen for co-formulations of basal insulin and GLP-1 agonists such as Xulthophy® and Lixilan.

Development of co-formulations to meet these demands, calls for new methods to analyse the active pharmaceutical peptides/proteins in solution. This poster shows how 1D diffusion NMR can be applied to investigate interactions between pharmaceutically relevant peptides under formulation conditions, i.e. in the presence of excipients (phenol, propylene glycol etc).

6. A Simple Route to Fluorescent Nucleosides via Purine Bis-triazolyl Derivatives

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Abstract:

Environmentally sensitive fluorescent nucleosides are attractive as reporter molecules. Herein we present the results of our research of purine nucleoside 6-amino-2-triazolyl derivatives that exhibit fluorescent properties and are readily available from reaction of corresponding 2,6-bis-triazolylpurine nucleosides with amines. The *N*-nucleophiles suitable for S_NAr reaction include even sterically

hindered secondary amines, hydrazines, amino acids and thiols. In addition, 2,6-bis-triazolylpurine nucleosides readily produce peptide-nucleoside conjugates.

The photophysical properties of synthesized fluorescent nucleosides were investigated. From our research in *ribo*-, *deoxyribo*- and *arabino*- series we concluded that the fluorescence properties of the obtained products are practically unaffected by sugar moiety. On the other hand, the emission spectra and quantum yields sensitively changed according to solvent properties. The solvatochromic properties of newly designed fluorescent nucleosides will be discussed.

The fluorescent adenosine analogue *N*⁶-methyl-2-(1,2,3-triazol-1-yl)deoxyadenosine (A*) has been incorporated in trinucleotide sequence d(CA*G). Although the quenching of fluorescence was observed due to the presence of other nucleobases, the developed trinucleotide still retain experimentally useful levels of fluorescence. Such result is encouraging to conduct further research to determine whether A* exhibits base-discriminating properties.

In summary, we successfully synthesized novel environmentally sensitive fluorescent nucleosides with promising photophysical properties.

7. The Development and Validation of QC Analytical Techniques for the Study of Peptides and Complex Peptide Mixtures

Dave Jardine and Alex McDowell, Tepnel Pharma Services

Abstract:

The development and validation of QC analytical techniques for the study of peptides and complex peptide mixtures is not trivial. In many cases, methods are either non-existent and require full development or are commonly not fit for purpose having been developed for research purposes and need re-evaluation and development before verification and validation.

Three case studies will be presented to highlight the difficulties of these projects. Case study 1 will demonstrate the development of a purity method for a complex mixture of 8 peptides; Case Study 2 the re-development of a method for peptide aggregations studies; and Case Study 3 will highlight issues raised during an OOS investigation during a Stability Program for a peptide product. Data will be presented to show improvements to methodology and the significant effects this had on each study.

8. Tumour Cell Targeting of Hyaluronic Acid Coated Nanoparticles for Nucleic Acid Therapeutics

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Abstract:

Targeted and efficient tumour cell internalisation is key to realise the benefits of nucleic acid (siRNA, miRNA, mRNA, DNA) based therapeutics in oncology. Optimum design of carriers avoiding non productive/lysosomal degradation and providing improved cytoplasmic bioavailability continues to be rate limiting in their successful progression to the clinic. Preclinical to clinical translation of ligand/receptor mediated targeting kinetics, receptor presentation and recycling rates and effect on

gene silencing/protein expression activities in diseased cells has been limited to date. Here we present physicochemical characterisation of 2 different nanoparticle systems designed to exploit the CD44 receptor pathway overexpressed in several tumours for delivering siRNA into tumour cells. Also presented are mechanistic insights into internalisation pathways, initial *in vitro* gene silencing, cytotoxicity and cellular stress data of an siRNA payload encapsulated into proprietary HA coated nanoparticles in specific tumour cell lines, with a view to systemic or local delivery of nucleic acid payloads for oncology therapeutics.

9. A New Class of DNA Nanoparticles as Potential Drug Carriers for the Treatment of Ophthalmic Diseases

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Abstract:

Currently diseases of anterior segments can only be treated with multiple daily doses of highly concentrated drugs. Treatment is severely hindered because only approximately 1-5% of the applied drug stays on the eye long enough to be effective. The major part is washed away by eye lid movement and tear fluid. Due to this inefficiency, high concentrations of the drug and frequent application of eye drops are required. This leads to side effects and a poor patient compliance. We have developed a drug carrier for eye drops based on a new class of nanoparticles (NPs) that have high affinity to the cornea. The NPs consist of micelles formed with DNA-strands of lipid-modified and conventional nucleotides. These NPs can easily be loaded with a large variety of drugs through different binding possibilities. In our proof-of concept study in rats the NP showed excellent binding to the cornea epithelium compared to pristine drug molecules. The NPs were even found up to 4h after application to conscious rats. Furthermore, preliminary in-vivo and in-vitro safety studies showed no negative effects.

10. Successful Development of an Algorithm for Antisense LNA™ GapmeR Design

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Abstract:

Exiqon develops strategies for the optimal design of single stranded antisense LNA™-enhanced gapmers (also known as LNA™ GapmeRs) that catalyze RNase H dependent degradation of target RNAs. We have developed an empirically derived design algorithm that provides Antisense LNA™ GapmeRs that achieve highly specific and potent target knockdown with a high hit-rate. Traditionally gapmers are designed against mature spliced transcripts. However a series of observations made us suspect that gapmers also target primary transcripts. Here we show that Antisense LNA™ GapmeRs targeting introns of an mRNA and a long non-coding RNA (lncRNA) are capable of potent silencing of the mature spliced transcripts. These observations have profound consequences for gapmer design and as a result we have modified our design algorithm, and we propose an alternative model for the mode of action of LNA™ GapmeRs.

11. Di- and Tetra-Lipid Derivatives of Oligonucleotides

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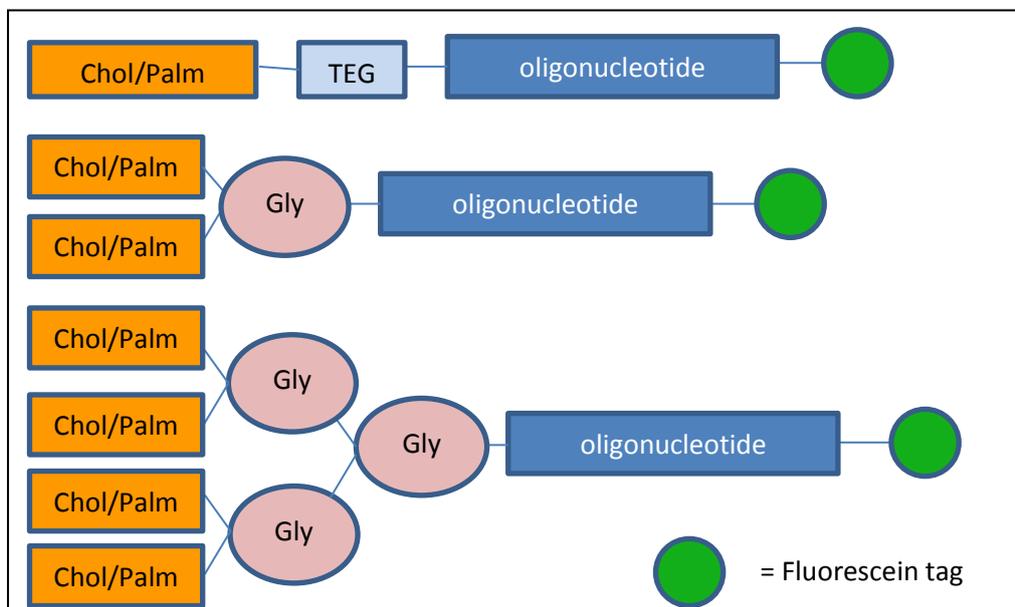
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Abstract:

Oligonucleotides, as gene specific tools are promising therapeutic or genome editing agents, but their broad *in vivo* applications are hindered by their low efficient cellular uptake. Several methods have been already developed to improve their delivery, but still there is no perfect solution. One of the promising ways is the synthesis of oligonucleotide-lipid conjugates.

Di- and tetra- dimethoxytrityl, cholesteryl and palmityl 5'-derivatives of 3'-FAM labelled oligonucleotides were synthesized and their lipophilicity was compared with HPLC analysis. The uptake efficiency of oligonucleotide di- and tetra-lipid derivatives were compared to mono- and unmodified ones into mammal and plant cells followed by fluorescence microscopy.



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