Principles of in vivo nucleic acid delivery with acylated cell-penetrating peptides

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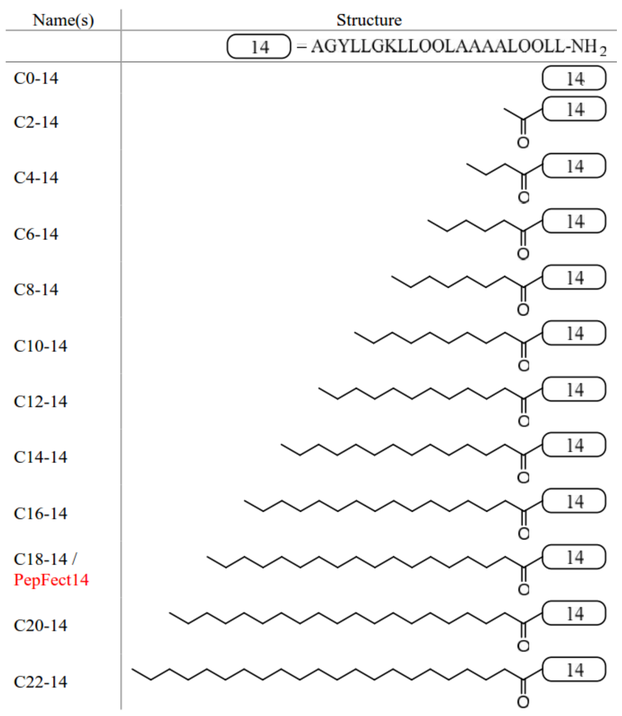
Introduction

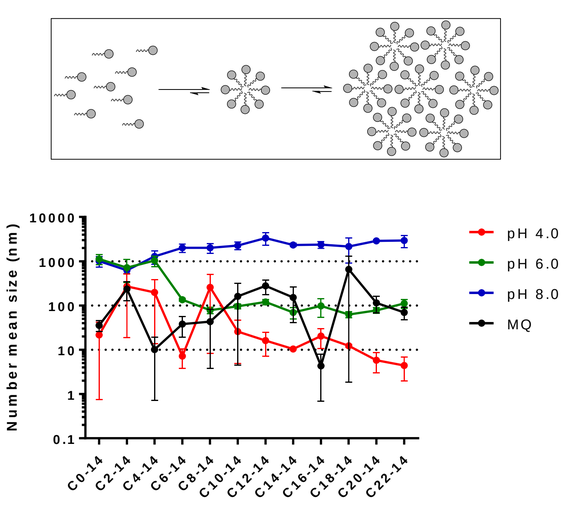
N-terminal acylation of cell-penetrating peptides (CPPs) with fatty acids is a well-known method to improve their ability to deliver non-covalently bound nucleic acid cargoes inside cells.
The aim of this study was to systematically describe the role of the N-terminal acyl chain length in translation of plasmid delivery from in vitro to in vivo.
We took a previously studied set of acylated CPPs with varying N-terminal acyl chain length (2 to 22 carbons) as the basis[1]. To evaluate the delivery efficiency, the peptides were non-covalently complexed with plasmid DNA and tested for gene induction both in cell cultures and in mice after systemic administration.

Conclusions

- Modifying CPPs with longer acyl chains improves the transfection efficiency of plasmid DNA both in cell cultures and in liver after systemic administration in vivo.
- CPPs with longer acyl chains form more stable nanoparticles upon non-covalent complexation of nucleic acids.
- Although free peptide fraction in the peptide/nucleic acid complexes is known to cause hemolysis and toxicity in vitro[1,2], these side-effects can be overcome in vivo by minimizing the amount of free CPP fraction in the complexes.

Self-association of the acylated peptides is dependent on protonation

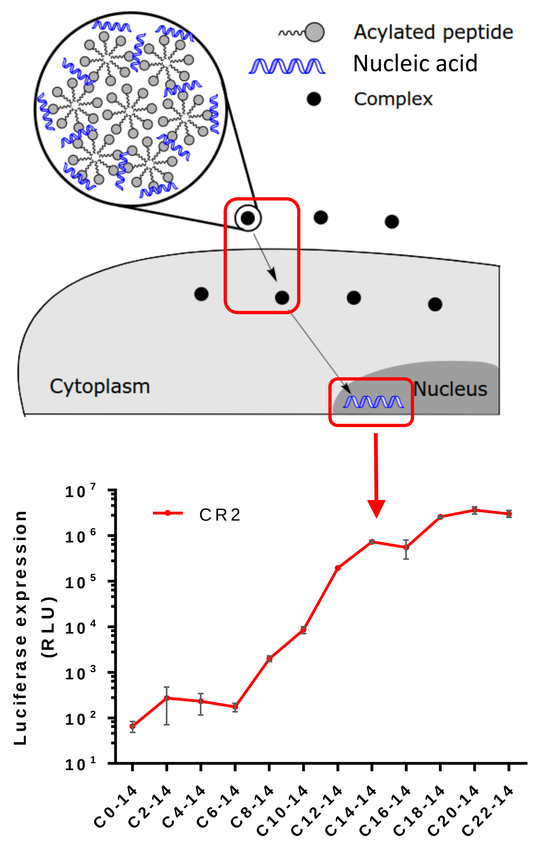




Self-association of peptides was studied by DLS. 10 µM peptide was diluted either in Milli-Q (MQ) or in 10 mM phosphate buffers at different pH.

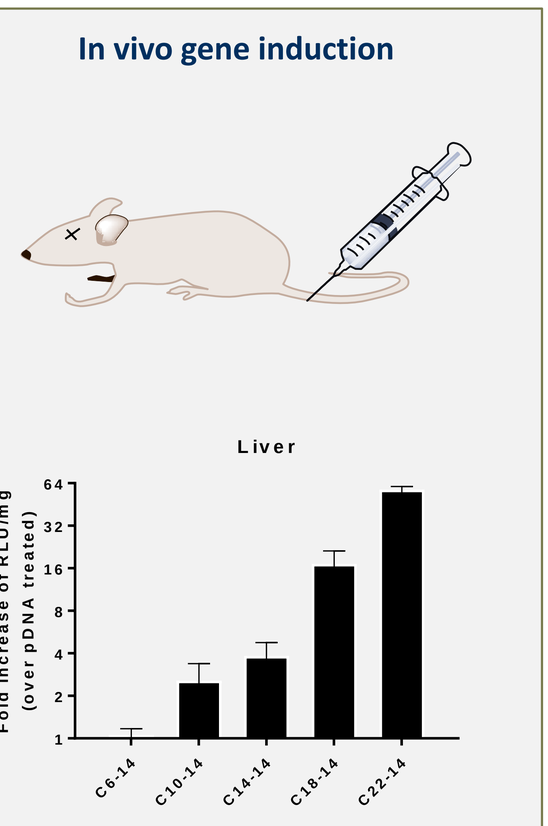
Transfection efficiency improves with longer acyl chains both in vitro and in vivo

In vitro transfection



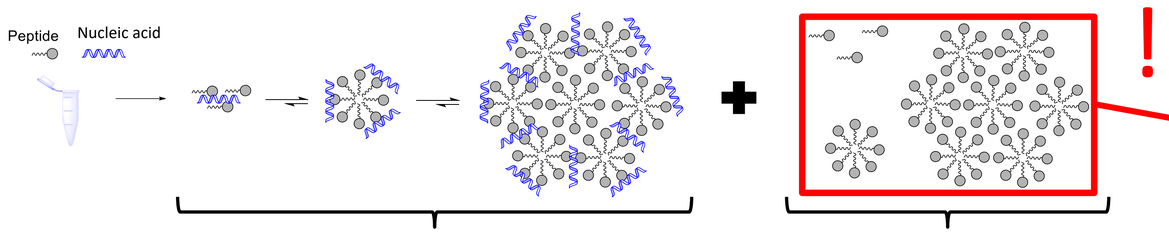
Transfection efficiency of complexes was evaluated in CHO cell line at 0.1 µg per well pGL3 plasmid dose after 24h.

In vivo gene induction



Gene induction in liver 24h after intravenous administration of 50 µg of pLuc2 plasmid complexed at CR2. (n=2-6 animals per group).

Formation of non-covalent peptide/nucleic acid complexes



Complexes are formed in Milli-Q at diferent [peptide:nucleic acid] charge ratios (CR).

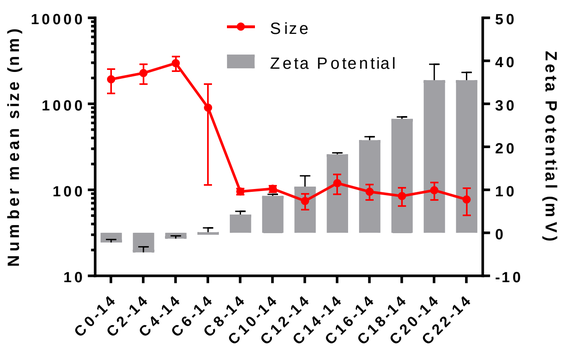
Toxicity of the complexes in vivo is related to the free fraction of peptide and acyl chain length

	CR2 50µg			CR4 20µg		
Peptide	**No of animals in group**	**Live after 24h**	**Survival Rate (%)**	**No of animals in group**	**Live after 24h**	**Survival Rate (%)**
C6-14	2	2	100	5	5	100
C10-14	4	3	75	7	1	14
C14-14	4	2	50	7	3	43
C18-14	6	6	100	9	7	78
C22-14	4	4	100	7	2	29

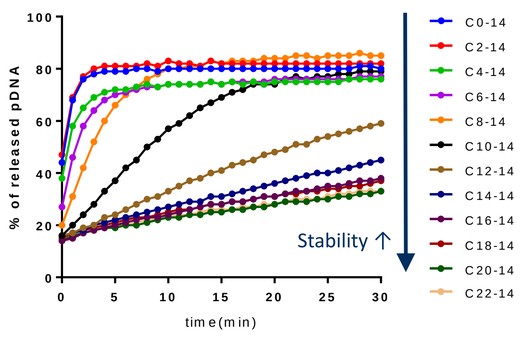
Number and survival of BALB/c mice in treatment groups 24h after i.v. administration of complexes at diferent charge ratios. All animal experiments and procedures were approved by the Estonian Laboratory Animal Ethics Committee (approvals no. 69 and 70, dated Feb 9, 2011).

Longer acyl chains induce more stable nanoparticle formation

A



B



The size and zeta potential of the complexes at CR2 (**A**) and stability to competitive polyanion displacement (**B**) were studied by DLS and PicoGreen assay respectively.

References

1. Lehto, T., Vasconcelos, L., Margus, H., Figueroa, R., Pooga, M., Hällbrink, M., and Langel, Ü. (2017) Saturated Fatty Acid Analogues of Cell-Penetrating Peptide PepFect14: Role of Fatty Acid Modification in Complexation and Delivery of Splice-Correcting Oligonucleotides. Bioconjugate Chemistry 28, 782–792.
2. Vasconcelos, L., Lehto, T., Madani, F., Radoi, V., Hällbrink, M., Vukojević, V., Langel, Ü. Simultaneous membrane interaction of amphipathic peptide monomers, self-aggregates and cargo complexes detected by Fluorescence Correlation Spectroscopy. Biochim. Biophys. Acta – Biomembranes (In press). doi:10.1016/J.BBAMEM.2017.09.024

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