#1503 - Enhanced transfection of oligonucleotides by modified peptide dendrimer vectors

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Background
Development of highly efficient and safe gene transfection reagents has become the essential factor for biological research and prospective successful application of gene therapy. Unfortunately, most transfection reagents have polymeric or complicated structures, moreover the reagents could be too toxic for in vivo use. Earlier[1], we reported peptide dendrimer (PD) LTP possessed both biocompatibility and good transfection efficiency, including in vitro and in vivo systems. But the peptide does not reach the Lipofectamine L2000 (Lf) level of transmembrane activity yet. Herein the aim was to work out the applicable approaches to transfection enhancement using such peptides.

Method
The PDs (LTP, D5, KK18, KK50) were obtained as pure products from standard building blocks by solid-phase peptide synthesis with the Fmoc-chemistry protocol using PS3 peptide synthesizer. The peptide purification after resin cleavage was conducted by preparative RP-HPLC with ODS. The PD structures were confirmed by MALDI-TOF MS. Further hydrophobic modification for synthesis lipid conjugated peptides (KK21, KK54, TA22) was carried out via condensation of maleimide-bearing lipid chain (palmitoyl-/caproyl-) to SH-group of the C-terminus cysteine residue. The cytotoxic properties were studied using HeLa HI and MA-104 cells. The in vitro gene transfer e□ciency was estimated in 293T cells by using the firefly luciferase reporter gene as a part of pGL3 plasmid and Lf as control.

Results
The level of cellular uptake of the PDs is impacted not only by the positive charge density of the peptide molecules, but as well by their conformations and membrane affinity. The ability to form the compact complex with oligonucleotides was improved by increasing lability of the binding cationic center of dendrimer through additional glycine residues after peptide branch points and reducing generation of PD. The insertion of non-polar aromatic residues close to C-terminus led to significant increase of transfection efficacy. Further hydrophobic modification resulted in transfection activity even higher than Lf. However directed lipid modification of LTP didn’t cause transfection enhancement.

Conclusion
PDs with high lability of cationic chains and appropriate level of amphiphaticity have ability to act as nontoxic single component ONs transfection reagents with equal or better efficiency than the standard Lf.

[1] Kozhikhova K.V. et al., *Org. Biomol. Chem.,* 2018, 16(43), 8181-8190.