

P029 Cellular delivery of nucleic acids by a non-covalently attached peptide carrier: quantitative analysis of cellular uptake and biological effect

Laufer, S.D., Recke, A.L., Trampe, A., Veldhoen, S. and Restle, T.

Institute of Molecular Medicine, UK S-H, Ratzeburger Allee 160, 23538 Lübeck, Germany

Various classes of nucleic acids have acquired growing interest as therapeutically relevant drugs. However, efficient *in vivo* delivery still remains a major obstacle. In this context, cell penetrating peptides (CPP) represent an innovative concept. In contrast to most CPP approaches which require a covalent linkage of carrier and cargo, the 27 amino acid peptide MPG α forms stable non-covalent complexes with nucleic acids, facilitating a maximal degree of flexibility. Applying either siRNA or antisense oligonucleotides, we performed detailed quantitative side by side analyses of cargo internalization and corresponding biological effect to determine the overall efficacy of each system. Using siRNA as cargo, reporter gene activity could be inhibited with an IC₅₀ in the sub-nanomolar range, requiring approximately 10000 siRNA molecules per cell. Delivery of a steric block oligonucleotide led to efficient splice correction with 50-100 \times up-regulation, requiring 7×10^7 steric block molecules per cell. Despite these encouraging results concerning the uptake of bioactive cargo, live cell fluorescence images and effects of various inhibitors on cellular uptake indicate that MPG α /nucleic acid complexes enter cells via an endocytotic pathway, with the majority of internalized cargo being trapped in vesicles. **Together**, our quantitative studies impressively show the limitation and at the same time the enormous potential of peptide-mediated nucleic acid delivery.