Effect of oligopeptide orientation on polymer-based DNA delivery

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Non-viral synthetic gene therapy reagents offer excellent structural and chemical versatility within non-immunogenic delivery systems, coupled with high therapeutic gene carrying capacity and long shelf life, making them attractive alternatives to viral systems. The success of non-viral transfection using polymers hinges on efficient nuclear uptake of nucleic acid cargo and overcoming intra- and extracellular barriers. This poster will describe the integration of the PKKKRKV heptapeptide (the Simian virus SV40 large T-antigen nuclear localization sequence, NLS) onto a polymer backbone, and the resultant high reporter gene expression in mice when administered by intramuscular ultrasound-mediated delivery. These novel polymers afforded protein expression higher than JetPEI™ *in vivo*, and in cell culture outperformed commercial reagents JetPEI™ and Lipofectamine 2000™, the latter being notorious for coupling high transfection efficiency with cytotoxicity. The orientation of the NLS peptide grafts relative to the polymer backbone markedly affected transfection performance both *in vitro* and *in vivo*. Quantitative polymerase chain reaction (qPCR) studies on transfected cells showed that polymers having the NLS attached at the valine residue afforded higher nuclear translocation, and subsequently higher protein expression, relative to those having the NLS groups attached in the opposite orientation. Besides nuclear uptake, the superior binding characteristics of these comb polymers compared to linear polylysine, as judged by atomistic and coarse grain simulations as well as polymer-DNA binding experiments, contributes to their enhanced transfection performance. Polyplexes formed from these comb polymer structures exhibit low cytotoxicity and high transfection efficiency both *in vitro* and *in vivo*, demonstrating the therapeutic promise of these novel gene therapy reagents.