SYNTHESIS, CHARACTERIZATION AND EVALUATION OF AMINOXY AND IONIZABLE LIPIDS FOR THE MODULAR ASSEMBLY OF LIPID-BASED NANOPARTICLES FOR EFFICIENT DELIVERY OF THERAPEUTIC NUCLEIC ACIDS

Dattatry Bhosale[a], Jihao Yu[b], Qinchen Zhang[b], Dongesheng Xie[b], Luboš Jelinek[c], Miroslav Havránek[c], Lei Fu[b], Andrew D. Miller[a,b,d,e,*]

[a]Veterinary Research Institute, Hudcova 70, 62100 Brno, Czech Republic
[b]School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P. R. China
[c]APIGENEX s.r.o., Podě bradská 173/5, Prague, 190 00, Czech Republic
[d] Institute of Pharmaceutical Science, King Collee London SE19NH, United Kingdom
[e]KP Therapeutics Ltd, 86 Deansgate, Manchester, M3 2ER, UK
[*] miller@vri.cz

Synthetic (non-viral) vectors, based on organic chemistry comprise synthetic and naturally available chemical components that assemble spontaneously with therapeutic nucleic acids to form synthetic nucleic acid delivery systems. These effect functional delivery of therapeutic nucleic acids to target cells *in vitro* and *in vivo*. Current clinical data, lipid-based nanoparticles (LNPs) are now the leading synthetic nucleic acid delivery system in mediating the functional delivery of small interfering RNAs (siRNAs) *in vivo* and in patients [1, 2]. Now there is needs to enhance research and depth of LNP possibilities for future clinical applications. For this reason, we have been developing a range triggerable LNPs. The LNPs are designed for stability in biological fluids, from points of administration target cells are reached at which point they are triggered to release their therapeutic agent in response to endogenous changes (e.g. pH, enzyme redox) or to some exogenously applied signal (e.g. light, thermal). Of particular interest to us over recent years have been pH-triggered LNPs for functional siRNA delivery *in vivo*, that are assembled and enabled by reversible click chemistry [3, 4].

Here we describe the efficient synthesis of three aminoxy_lipids; cholesteryl aminoxy lipid (CA-1), cholesteryl TEG-aminoxy lipid (CTA-2), and cholesteryl-PEG^{350}-aminoxy lipid (CPA-3), followed by their formulation into pH-triggered LNPs for functional siRNA delivery *in vitro* and *in vivo* with novel PEG_2000 aldehyde variants. Also, the synthesis of two ionizable lipids (DODAG-1, CDAN-2) [4] that have already proven to have utility *in vivo* as key compartments of LNPs and are expected to form the basis of new clinically relevant ionizable lipid families competitive with those recently described from commercial sources. Finally, we outline the results of the biophysical studies with pH-triggered and other LNPs to investigate the effects of temperature, ionic strength and pH on LNP structure and structural integrity, providing important information for the future design and creation of such LNPs for highly efficient functional delivery of therapeutic siRNAs to liver cells *in vivo*.

The authors acknowledge the financial support from the OPVVV project FIT EN.02.1.01/0.0/0.0/15_003/0000495.

[1] Tseng, Y. C. et.al.; Adv. Drug. Delivery Rev., 2009, 61, 721-731.

[2] Semple, S. C. et.al.; Nat. Biotechnol., 2010, 28, 172-176.

[3] Carmona, S. et.al.; Mol. Pharmaceutics, 2009, 6, 706-717.

[4] Kolli, S. et.al.; Biconjugate Chem., 2013, 24, 314-332.