ESRF	Experiment title: **Towards Effective Non-viral Gene Therapy: SAXS studies on novel rigid gene delivery vectors (continuation of MX-1607)**	**Experiment number**: MX-1713
Beamline:	**Date of experiment**: from: 17th June to: 18th June 2015	**Date of report**: 25th November 2015
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These SAXRD/SAXS measurements are part of a collaborative, interdisciplinary nonviral gene therapy project, and are a continuation of those carried out previously using ESRF beamtime (MX-1485 and MX-1713). The results from these beamtime allocations are described in five peer-reviewed papers [1- 5]. The experiments are part of a wider study on lipid-DNA particles that includes the complementary techniques, laser-scanning confocal microscopy used to probe the interactions of cationic lipid DNA particles with cells, and by luciferase reporter-gene expression assays which will measure transfection efficiencies in mammalian cells.

Gene therapy is the process of replacing a non-functional segment of DNA with functional DNA. Since free DNA cannot cross the cellular membrane on its own, a delivery agent or vector is required to facilitate this (transfection). There exist two general vector systems in use today: viral and nonviral. Transfection efficiency with nonviral vectors is still significantly low compared to gene delivery using viral vectors. However, patient deaths during clinical trials in the 1990s using viral vectors resulted in the resurgence of nonviral studies. Work (including results from proposals MX 1485) [1] focuses on the effect that lipid shape has on the super-molecular ordering of the lipid-DNA complex (lipoplex) phase (typically lamellar or inverted hexagonal), size, and ultimately transfection efficiency. Lipoplex phase and size matter in gene delivery. The next phase (proposal MX 1607) involves working towards the goal of establishing a universal structure-function relationship [4, 5] that defines effective nonviral delivery systems. This is important because testing novel structures is largely empirically driven. Rational vector design is necessary to the development of nonviral gene delivery strategies. Through correlations between the shape of lipid/DNA complexes (as determined by the SAXD analyses) and their associated in vitro transfection efficiencies, this proposal aims to advance further our previous work [1 - 5] towards the rational design of lipid vectors followed by systematic lipoplex formulation guided by an hypothesis-driven justification for the impact of lipid shape on lipoplex morphology.

During the experiment we measured two series (8 + 12 = 20) of different lipoplex compounds as a function of temperature from 15 to 45 °C, in steps of 5 °C). In total 140 different lipoplex samples were

measured. Including the measurements on the relevant buffers, around 300 samples were characterised during the experimental session. Some classes of lipoplex samples showed indications of thermal induced phase transitions from lamellar (low temperature) to hexagonal packing (high temperature). Furthermore, an interesting thermotropic effect was also discovered for one of the (hexagonal) samples. The full analyses of these features are in progress [5].

References

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[3] Jubeli, E.; Maginty, A.B.; Khalique, N.A.; Raju, L.; Abdulhai, M.; Larsen, H.; Nicholson, D.G.; Pungente, M.D.; Goldring, W.P.D. Next generation macrocyclic and acyclic cationic lipids for gene transfer: Synthesis and *in vitro* evaluation (*in progress).*

[4] Parvizi, P.; Jubeli, E.; Raju, L.; Khalique, N.A.; Almeer, A.; Allam, H.; Al Manaa, M.; Larsen, H.; Nicholson, D.; Pungente, M.D.; Fyles, TM. Synthesis and study of pyridinium based cationic lipids as gene delivery vectors. 248th American Chemical Society National Meeting & Exposition, August 10-14, 2014, San Francisco, CA.

[5] Khalique, N.A.; Parvizi, P.; Jubeli, E.; Larsen, H.; Nicholson, D.G.; Pungente, M.D.; Fyles, T.M. Correlation of lipoplex morphology with transfection efficacy and cytotoxicity for pyridinium-based cationic lipids by means of synchrotron small angle X-ray diffraction (*in progress).*