| ESRF | Experiment title: **Towards Effective Non-viral Gene Therapy: SAXS studies on novel rigid gene delivery vectors (continuation of MX-1713)** | **Experiment number**: MX-1811 |
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| **Beamline**: | **Date of experiment**: from: 30th April to: 1st May 2016 | **Date of report**: 25th November 2016 |
| **Shifts:**3 | **Local contact(s)**: Petra Pernot | *Received at ESRF:* |

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This set of experiments builds on previous SAXS/SAXD studies (MX-1485/1606/1713) on lipid-DNA particles the measurements being part of a collaborative, interdisciplinary nonviral gene therapy project. Of the available ESRF beamlines, BM29 has proved to be eminently suited to our series of studies. As with all of the preceding measurements, the beamline performed well and the beam itself was stable. We were therefore able to make full use of the instrument. The collective results from the beamline allocations are described in six peer-reviewed articles [1 - 6].

Taken together, the overall results represent a significant progression in yielding structural information and this enables us to move forward in our quest for efficient transfection into cells. The experiments are an integral 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. The selected lipids are partly characterised by varying degrees of flexibility and this property appears to be an important factor in transfection.

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. One of our aims is to establish 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 in developing effective nonviral gene delivery strategies. It is by

the guidance of hypothesis correlating the shapes of lipid/DNA complexes (SAXD analyses) and associated in vitro transfection efficiencies that we aim to further advance our previous work [1 – 6].

During the experiment we measured two different lipid series (containing 5 and 3 lipids, respectively), each with two different co-lipids (giving a total of 16 unique liposomes formulations). In addition to these liposome formulations alone, plasmid DNA was formulated with each liposome (giving rise to lipoplexes), and these formulations were also studied. All samples were analysed as a function of temperature from 20 to 40 °C, in steps of 5 °C. In total 160 different lipoplex samples were measured. Including measurements on the relevant buffers, approximately 355 samples were characterised during the experimental session (which is a new "record" for our team in terms of output/unit shift). Some classes of lipoplex samples clearly showed thermal induced phase transitions from lamellar (normally at "low" temperature) to hexagonal packing (normally at "high" temperature). The full analyses of these features are in progress.

Dissemination of this study is currently covered by papers in progress. Furthermore, the results have led us to consider adding complementary techniques, such as SANS, and show that there is a need for future experiments at BM29. The work summarised here will form the basis for our next proposal for beamtime.

## References


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