ESRF	Experiment title: **Towards Effective Non-viral Gene Therapy: SAXS studies on novel rigid gene delivery vectors (continuation of MX-1485)**	**Experiment number**: MX-1607 on BM 29
Beamline:	**Date of experiment**: from: 14th May to: 15th May 2014	**Date of report**: 27th October 2014
Shifts:	**Local contact(s)**: Mathew Bowler	*Received at ESRF:*

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Report:

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). The results from that beamtime are described in two peer-reviewed papers [1,2]. The project is part of a wider study on cationic lipid (CL)-DNA particles that includes the complementary techniques laser-scanning confocal microscopy, in order to probe the interactions of CL-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 resurgence towards the refinement of nonviral strategies. Work (which includes results from 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 (MX 1607) involves working towards the goal of establishing a universal structure-function relationship that defines effective nonviral delivery systems. This is important because the testing of 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 SAXS analyses) and their associated in vitro transfection efficiencies, this proposal aims to advance further our previous work already accomplished [1,2] 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.

We have used the small-angle scattering (SAXS/SAXRD) facilities of BM29 (SAXS) to gain detailed structural information on a series of novel cationic lipid-DNA complexes. This information is required to

rationalise the structure-function relationships that are a prerequisite for designing more efficient lipid gene delivery vectors for effective gene therapies. The hydrophobic component, together with the positively charged head group associated with cationic lipids allows complex formation with the negatively charged DNA thereby resulting in lipoplex particles. A lipoplex particle that is neutral (or with a net positive charge) is able to pass through the cell membrane to ultimately release the DNA cargo within that cell. It is important to establish the relationship between structure and function of the lipid, and hence of the lipoplex, which is in part where the SAXS method comes in.

In total, SAXS/SAXRD data was acquired on 86 samples of lipid/DNA complexes prepared from a selection of our lipids during the allocated beam time. The data allow us to obtain low-resolution 3-dimensional structures in a natural environment. The quality of the data (depending on the sample characteristics) was overall good, and the data pre-treament greatly facilitated the posthumous analysis – focusing on the diffraction features arising from the multilamellar packing. The interesing q-range for the compounds in questions is $(0.5 < q < 3)$ nm^{-1}, meaning that a more narrow scan range may be forseen for future experiments.

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RESULTS:

Pyridinium-based Lipids: A class of pyridinium-based cationic lipids was synthesized bearing C9 to C22 saturated, unsaturated straight and branched hydrocarbon chains. The expectation was that these compounds representing a range of values of lipid shape parameter was expected to control the lamellar-hexagonal phase balance in complexes with DNA. The lipid phase behavior of the lipoplexes was derived from small-angle X-ray scattering experiments and correlated with the lipid shape parameter computed from molecular structure. The effect of variation in structure and lipoplex formulation on DNA transfection efficiency (as determined by β-galactosidase and GFP expression upon in vitro transfection) and cytotoxicity of these lipids in mixtures with co-lipids was assessed. These studies were applied to define a Transfection Index (TI) that encompasses the variation in the lipid shape parameter, the phase packing and the partition of these lipids into the lipoplex. Furthermore, the cytotoxicity of the lipid formulations was also correlated with the shape parameter and the partition of the lipids together with the experimental charge ratio used in lipoplex formation. Refinement of correlations between the biological outcomes (TI and cytotoxicity) and the lipid shape parameter are ongoing, and the preliminary findings were presented in an oral presentation at the 248th American Chemical Society National Meeting & Exposition (August 10-14, 2014, San Francisco, CA) [3], and are anticipated to be submitted in the form of a manuscript for peer-review [4].

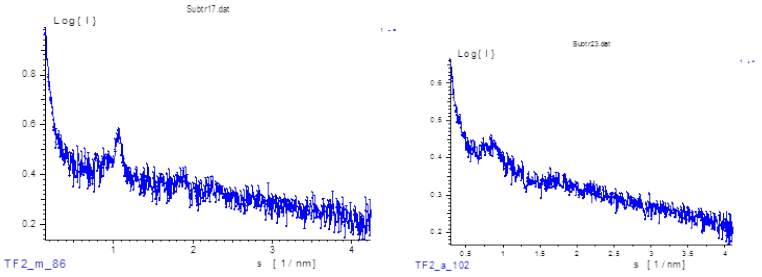

Figure 1. Small angle X-ray diffraction curves for two different pyridinium-based lipid/DNA lipoplexes. The one on the left shows hexagonal packing, whereas the one on the right shows lamellar packing features.

Macrocyclic and Acyclic Lipids: A series of cationic lipids which possess either a macrocyclic or acyclic hydrophobic domain based on C7 or C11, saturated or unsaturated chains were studied to probe the role that lipid structure and lipoplex formulation have on transfection efficiency, cytotoxicity and lipoplex phase behaviour. The differences in conformational rigidity between the hydrophobic domains of the macrocyclic and acyclic cationic lipids were expected to be reflected in both the relative ability of these lipids to protect the DNA in the lipoplex formulation (as determined by a DNase I gel-based degradation assay) and relative transfection efficiencies. Furthermore, we aimed to understand what impact the structural differences in the lipid hydrophobic domains might have in terms of lipoplex phase behaviour, as derived from small-angle X-ray scattering experiments. The measurements performed on these samples revealed a number of lipid/DNA

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complexes with very well ordered assembly, and all exhibited lamellar packing with a packing parameter ranging from 66-70 Å. The preliminary results were presented at the 66th Irish Universities Chemistry Colloquium (June 18-20, 2014, Galway, Ireland) [5], and a manuscript which describes a full account of this work is currently in preparation for peer-review [6].

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