ESRF	**Experiment title:** **Towards Effective Nonviral Gene Therapy: Synchrotron Small-Angle X-Ray Scattering Studies on Novel Rigid Gene Delivery Vectors**	**Experiment number**: MX-1485
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Shifts: 3	**Local contact(s)**: MUELLER DIECKMANN Christoph	*Received at ESRF:*

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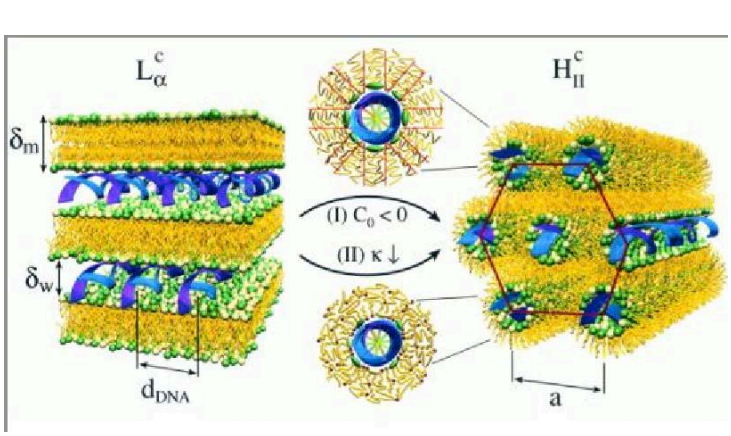
Report:

Gene therapy refers to the use of nucleic acids as a potential therapeutic treatment of a variety of diseases, including cancer and inherited disorders such as cystic fibrosis and cardiovascular disease. It involves replacing nonfunctional segments of DNA with functional DNA. Free DNA cannot passively and efficiently cross cellular membranes without the assistance of a delivery agent or vector. These facilitation processes are known as transfection and may be viral or nonviral. Unfortunately, positive results using viruses as the vehicle to carry the therapeutic gene have been overshadowed by pathogenic effects and a few deaths. This has given impetus to studies on nonviral deliveries as an alternative and this is the motivation for this experiment.

The overall aim of this experiment is to use synchrotron small-angle scattering (SAXS) to gain detailed structural information on a series of novel cationic lipid-DNA complexes (lipoplexes). This information is required to rationalise structure-function relationships. This is necessary for designing more efficient lipid gene delivery vectors that are required for effective gene therapies. Since cationic lipids have a positive charge they complex with the negatively charged DNA thereby forming a lipoplex. The charge means that the lipoplex is able to pass through the cell membrane to release its load of DNA within that cell. It is important to establish the relationship between structure and function of the lipoplex which is where the SAXS method comes in.

The facilities of the BioSAXS beamline (BM29) were used to collect SAXS data on 100 samples of the liposomes* and lipoplexes** prepared from a selection of our lipids. The data allow us to obtain low resolution 3-dimensional structures in a natural environment.



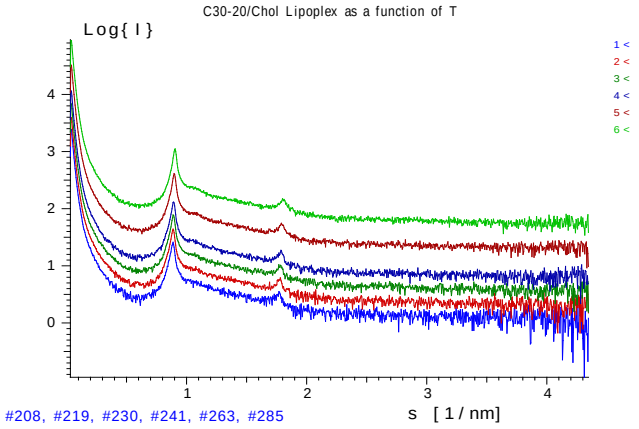


Figure 1 [left] *Conceptual depictions of cationic liposome-DNA complex (lipoplexes). DNA helices are blue. The formation of a layered multilamellar lipoplex (lft) or a hexagonal lipoplex (right depends on the type of cationic lipid used (Koltover, I et al Science, 281, 1998, 78.)*

Figure 2 *Small angle X-ray diffraction curves for the novel C30-20/Chol Carotenoid lipoplex for different temperatures. The lower (blue) curve shows the result for T = 20˚C, then each new at a temperature increase increment of ΔT = 5˚C. The upper curve is at T = 55˚C. Well defined multilamellar ordering is observed over the whole temperature range. The repetition distance remains quite constant over the temperature interval. A small decrease in δ as the temperature is increased is however noted: δ(T = 20˚C)= 71.1 Å to δ(T = 55˚C)= 69.7 Å. The curves are shifted along the ordinate axis for clarity.*

*liposome - a nanosized spherical sac of phospholipid molecules enclosing a water droplet (a micelle), which can carry drugs or other substances into the tissues.

** DNA can be covered with lipids in an organised structure such as a liposome (micelle).

Results

Prior to these measurements, it had been suggested that a pH-induced transformation of a cationic lipid-DNA complex from the lamellar to the inverted hexagonal phase within the endosome is responsible for the release of the DNA cargo [1]. We started the experiments partly because of our belief that the lipoplex structural data obtained from SAXS experiments with these novel lipids acquired over a pH-gradient will provide data on the arrangement of the DNA in the lipoplex, the lamella structure and the pH at which the DNA cargo is released as well as possibly some indication as to the structural changes during the process of release. The results demonstrate nicely the diffraction effects that are manifested as discrete peaks at certain angles that arise either from a layered packing arrangement or a hexagonal arrangement (Fig. 1). Simply put, this means that, in effect, we are seeing powder diffraction from a solution. This is rather unusual in a bioSAXS experiment and can be appropriately termed small angle x-ray diffraction or (SAXRD). The positions of the diffraction peaks enable us to unequivocally decide which of the two structures applies here and the corresponding characteristic repetition distance (being $\delta_w + \delta_m$ in the case of L_α^c or a in the case of H_{II}^c as given in figure 1). Data processing and analysis are ongoing.

The way forward from here is to correlate with in vitro transfection efficiency data in the hope that we can reveal lipid structure-function relationships. Ultimately, we wish to identify a refined and more effective gene delivery vector.

References

1. Bell, P.C.; Bergsma, M.; Dolbnya, I.P.; Bras, W.; Stuart, M.C.A.; Rowan, A.E.; Feiters, M.C.; Engberts, J.B.F.N. J. Am. Chem. Soc. 2003, 125, 1551.