ESRF	**Experiment title:** Investigation of the Mechanism of Formation of a DNA-Neutral lipid Complex using high Resolution Small Angle X-ray Scattering.	**Experiment number**: SC1040
Beamline: ID02A	**Date of experiment**: from: 05/09/02 to: 07/09/02	**Date of report**: 01/09/03
Shifts: 8	**Local contact(s)**: Dr. Stephanie Finet	*Received at ESRF:*

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Report:

All 8 shift, 72 hours were used for experiments. This time was divided into:

- Set-up time, calibration.
- Stopped flow kinetics experiments on DNA, DPPC calcium complex formation
- Static SAXS on a number of complexes, to evaluate kinetics on a much longer timescale (4 months)

The stopped flow experiments were carried out in uniform mode, at detector distances 1m and 1.5m and the detector used was an image intensified fast CCD camera. The timescales used for experiments were from 240ms after mixing to 1 hour after preparation. The complexes investigated were composed of calf thymus DNA, DPPC unilamellar vesicles prepared by sonication and calcium at varying concentrations. Individual components were added to each syringe. DNA and DPPC vesicles were mixed first, stored in the delay line for a short period and then calcium was added to this mixture in the cell (dead-time of mixing 100ms). All scans taken had a duration of 100ms. No evidence of lipid degredation was observed after 30 scans at this exposure. This was used as the number of scans for each individual experiment thereafter, regardless of the length of the experiment.

It was possible to track the evolution of complex formation using the stopped-flow mixing method (figure 1, top left). The lack of suitable theoretical models, makes the interpretation of the results difficult. It appears that initial binding of lipid to DNA via calcium takes place in the period before initial measurements are taken. The lamellar repeat distance for DPPC is ~6.3nm, whereas the first recorded d spacing observed was ~ 6.85nm. The increase in lamellar repeat distance after this, eventually reaching the lamellar repeat distance observed for equilibrated complexes prepared at this composition (figure 1, top right).

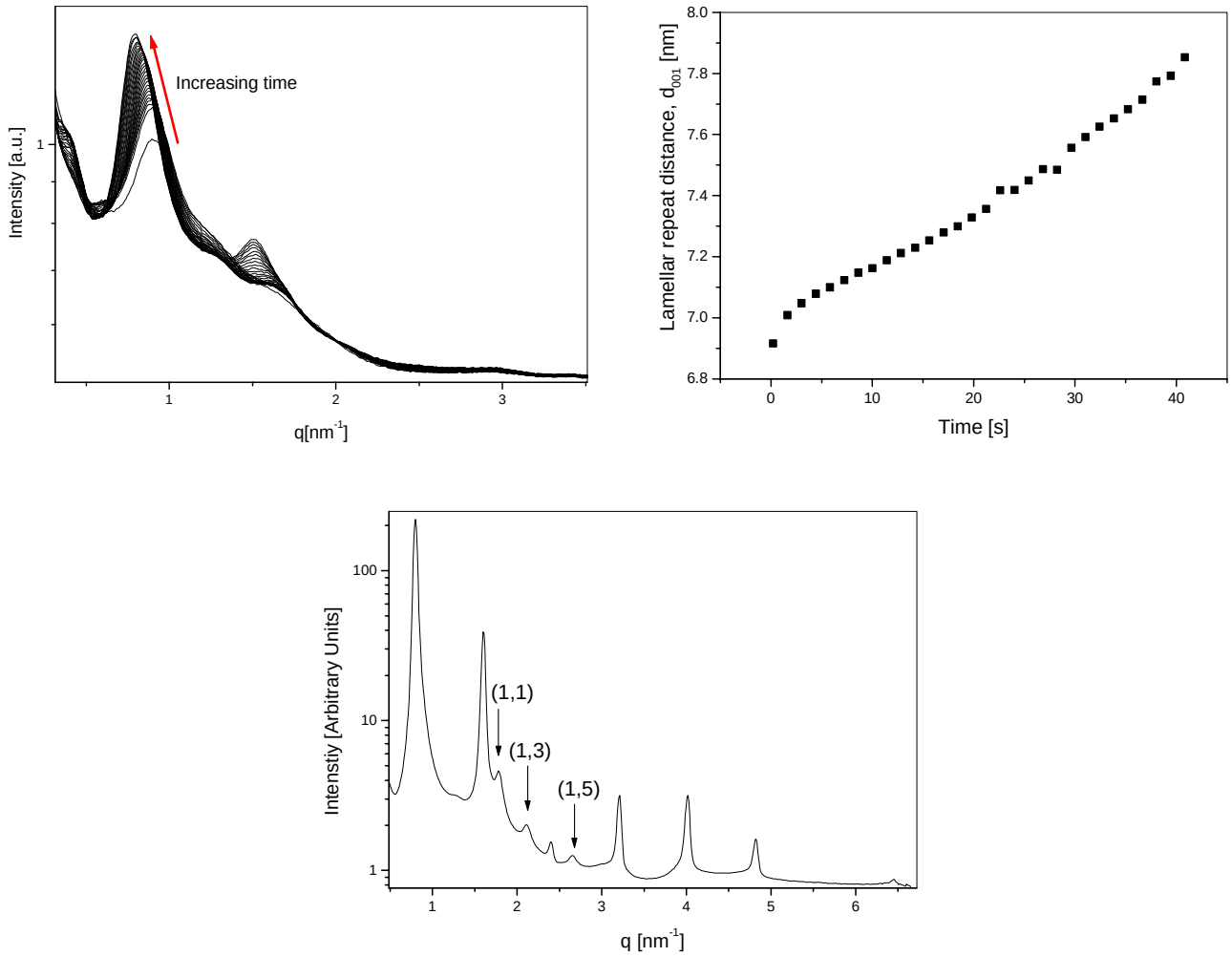

Figure 1: (Top left) SAXS patterns as a function of time for complexes prepared from unilamellar vesicles of DPPC, with DNA at a DNA:lipid mole ratio of 1:2 at a calcium concentration of 50mM. The timescale for this experiment was 240ms after initiating mixing to 40s after mixing. The lamellar repeat distance for the complex reaches the value obtained in equilibrated samples in this time-frame. (Top right) Lamellar repeat distance vs time for the complex described above. (Bottom centre) SAXS pattern for a complex prepared at a DNA:lipid mole ratio of 1:4 in 10mM $CaCl_2$, 4 months after preparation. The DNA peak corresponding to the DNA-DNA in plane correlation observed two weeks after formation is replaced by three new peaks, corresponding to the (1,1), (1,3) and (1,5) Miller indices of a DNA rectangular columnar phase.

Static experiments were carried out at a detector distance of 1m. Samples investigated were prepared 4 months prior to visit to ESRF and had been initially examined by SAXS, 2 weeks after preparation. No evidence of any change of multilamellar structure characteristics or lipid organisation were observed, however changes in DNA orientation were seen. This was not observed in the earlier investigations. A rectangular columnar phase of DNA was observed for a complex prepared at a DNA:lipid ratio of 1:4 in 10mM $CaCl_2$ (figure 1, bottom centre). The first reported DNA rectangular columnar phase for synthetic complexes, was reported for a mixture of cationic and neutal lipid by Artzner et al. [1].

From the results shown here and those taken over a longer period of time it appears that there are at least four steps involved in complex formation a) binding, which takes place in the millisecond time scale, b) lipid reorganisation (possibly fusion) on the DNA surface, increasing the lamellar repeat distance to the level usually observed in equilibrated samples ~7.8nm (millisecond – second timescale), c) multilayer formation (minutes timescale) d) DNA ordering (days - weeks (possibly months) timescale).

[1] Artzner F., Zantl R. Rapp G., Rädler J.O., (1998), *Phys. Rev. Lett.* **81**, 5015.