ESRF	**Experiment title:** Actin/Lipid composite Phase : Liquid Crystal Organisation	**Experiment number**: SC 938
Beamline: ID2	**Date of experiment**: from: 3 july 2002 to: 6 July 2002	**Date of report**: 1 march 2003
Shifts: 9	**Local contact(s)**: T Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Franck ARTZNER, Céline Valery, Cécile ALLAIS, Aurélie PARETTE
UMR 8612 (CNRS), Faculte de pharmacie
Chatenay Malabry, FRANCE
Anne RENAULT
Université de Rennes
FRANCE

Report: The wavelength energy was 12.0keV with a the beam a size of 200x200μm^2 on sample and on detector. Attenuation was only due to the beam definition slits. Two detector were used, the CCD and the Image Plate. In the case of the CCD detector, the sample/detector distances were 0.9m, 1.5m, 6m. In this condition, the accessible scale was q=0.005-0.7Å^{-1}, and the resolution is due to the detector pixel resolution (200/400μm). The 9 shifts, 72 hours, were used as described in the following :

A] beamline alignment, setup installation and tests.

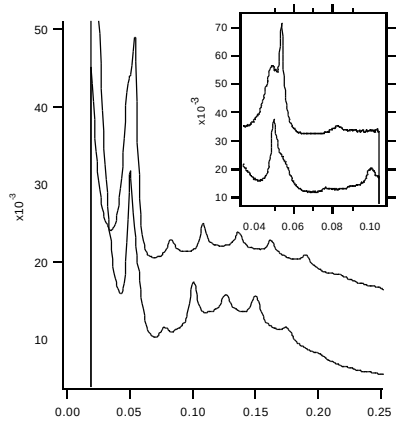
B] sample stability tests. Under these conditions, actin samples are stable only with pulse shorter than 100ms. Radiation damages are observed after 500ms. This was clear in the case of the Image plate tests with actin complexes, in which cases, a systematic diffraction degradation as observed.

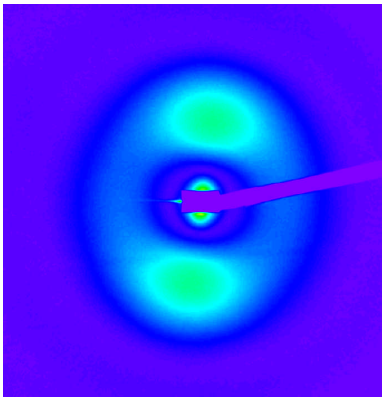
C] Inhomogeneous samples Actin/cationic lipid complexes were investigated at temperature ranged between 10 and 50°C. The preliminary results were confirmed and new results were obtained.

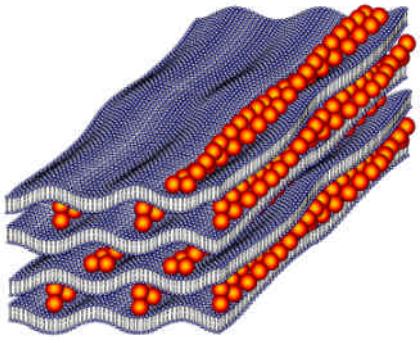
1) The 3D structure with corrugated membranes was fully characterized on 15 samples in both fluid or gel state of the lipid chain (for an example fig. 1). This demonstrate the initial structure proposition and indexation in an orthorhombic cell with three large parameters (Fig. 2) : a=35nm (pitch along the actin filament), b = 24nm (thickness of 2 lipid bilayers + 2 actin filaments), c = 15-23nm (interdistance between actin filaments)

2) A new 2D structure was characterized by X-ray diffuse scattering on aligned samples (Fig. 3) and freeze fracture electron microscopy on the same samples. The structure is constituted by isolated lipid bilayers on which are electrostatically adhered actin filaments (Fig. 4).

3) The dimensionality of the structure (2D or 3D) is correlated with the macroscopic stiffness of the fiber, and are relevant for comprehension of mechanical properties of Actin filament bundles of cells.







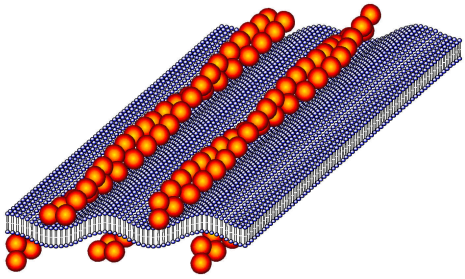


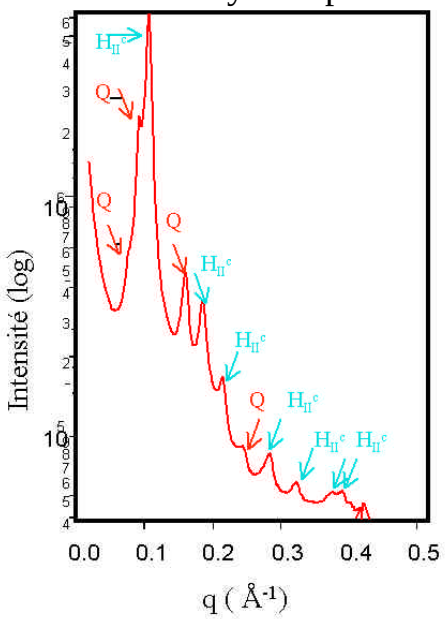
Fig. 1 (left) : SAXS at 1.5m and 6m (inset) at low (bottom) and high (top) temperature. The diffuse scattering due to actin position changes during the transition.

Fig. 2 (right) : 3D structure of Actin filaments embedded within cationic lipid bilayers

Fig. 3 (left). Diffuse scattering of aligned samples, characteristic of an isolated membrane.

Fig. 4 (right) 2D structure of Actin filaments adhered on isolated membrane.

D] DNA/lipid complexes were prepared in pH gradient in capillaries. These capillaries were measured at 25 positions corresponding to distinct pH, in an home-made oven for 20 samples. In this condition 25 pH of 80 samples were studied. The data were analyzed automatically by an home-made program. We demontrate that during a pH increase, the lamellar complexes of DNA/DOPE/DOTAP (the most classical lipid mixture used in gene therapy) release the DNA and transform into an lipid hexagonal phase, via a coexistence of a lipid bicontinuous cubic phase and a hexagonal phase of DNA complexes. The first observation of an intermediate cubic phase should be the key to explain the gene therapy efficiency of this lipid mixtures.



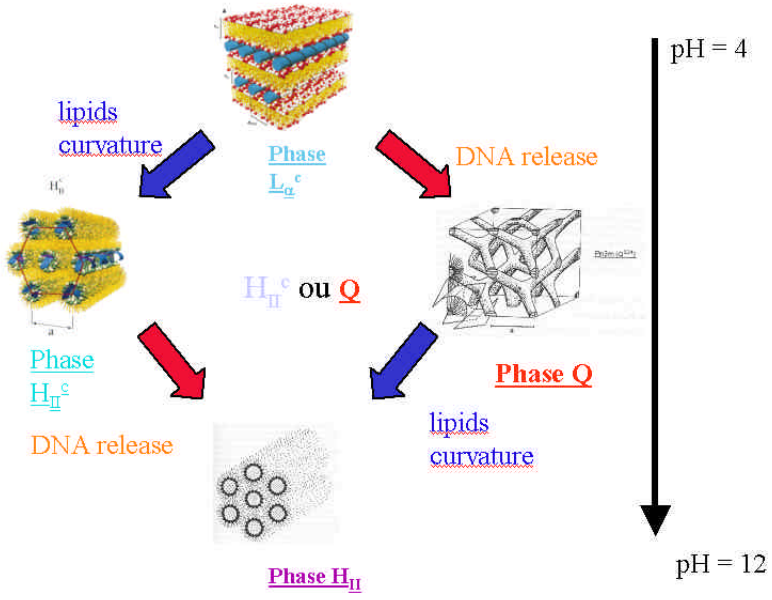


Fig. 5. SAXS of intermediate states demonstrating the coexistence of a cubic phase (Q) of pure lipids and an hexagonal phase (H_{II}^{C}) of DNA/lipid complexes (left). Structural evolution of the complexes induced by a pH increase. Note the presence of two intermediate states.