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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

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Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

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All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** **Supramolecular ordering of nucleosome core particles: polycations and tail effects**	**Experiment number**: SC1819
Beamline: ID02	**Date of experiment**: from: 28 october to: 31 october 2006	**Date of report**:
Shifts: 9	**Local contact(s)**: Stéphanie Finet	*Received at ESRF:*

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Report:

Despite the recent crystallographic works on nucleosome core particles (NCPs) and tetranucleosomes, the structure and supramolecular organisation of chromatin remains unsolved. In our approach, we decided to work on a simplified model system consisting of isolated nucleosome core particles. Previous works have shown that NCPs prepared from native chromatin display a large variety of supramolecular organisations when the monovalent salt concentration and the osmotic pressure of the sample are modulated. Our present work had two goals. Firstly, we planed to understand the effect of the divalent and trivalent ions on the supramolecular organisation of nucleosomes. And secondly, we intended to emphasize the role of the histone tails in this organisation.

1- We restricted our work to the effect of magnesium (Mg 2+) and spermidine (Spd 3+).
The addition of these multivalent cations induces the aggregation of NCPs. The aggregates were placed in cylindrical capillaries. Around 60 capillaries were scanned by the X-ray beam. Changing the temperature from 20°C to 37°C gave rise to macroscopical changes of the capillary aspect; precipitates were transparent at 20°C and turned white at 37°C. For this reason, all X-ray diffraction patterns were recorded at 4°C, 20°C, and 37°C.
The phase diagram of NCP precipitated with spermidine (Spd 3+) is displayed in Figure 1. Just above the precipitation threshold X-ray patterns show a stacking of NCP which progressively leads to the formation of columns by increasing the spermidine concentration. Near the maximum level of precipitation, narrow Bragg peaks are observed which are characteristic of a 2D columnar hexagonal ordering. For higher spermidine concentration this 2D ordering is destroyed and all that remains is the NCP stacking which progressively disappears by increasing the spermidine concentration. Near the redissolution threshold, NCPs present an isotropic order.
The parameters of the 2D hexagonal phase have been determined. The NCP concentration inside the dense phases was estimated to vary from 500 to 600 mg/ml.

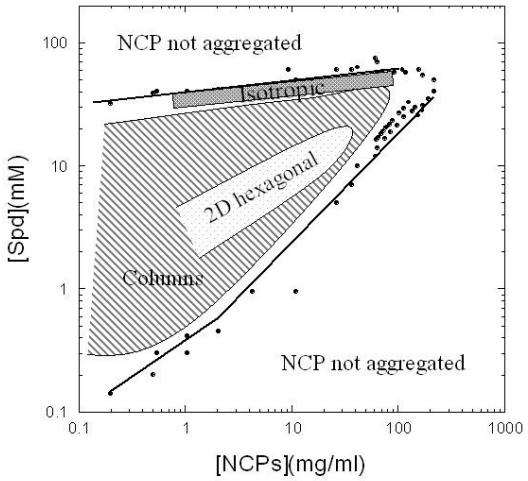

Figure 1: Phase diagram of NCP in the presence of spermidine (Spd 3+) as a function of NCP and trivalent ion concentrations. Temperature does not affect the organization between NCP, but decreasing the temperature gives rise to an small increase of the parameters.

Upon aggregation with Mg2+ cations, NCP are poorly ordered. We only observed an isotrophic phase of isolated nucleosomes and a poorly ordered columnar phase that could be either isotropic or nematic. The effect of magnesium ions has also been studied under non-aggregating conditions. The dense phase is then formed by addition of a solution of the stressing polymer PolyEthyleneGlycol (PEG). Dilute solutions of NCP (with given Na+ and Mg2+ concentrations) were placed at the bottom of a capillary, and the PEG solution (prepared in the same salt solution) was added on top of it. In the present work, the monovalent salt concentration ranged from 10 to 160 mM, the magnesium concentration from 1 to 100 mM and the PEG concentration from 13% to 35%. A total of 150 capillaries were prepared and scanned with the X-ray beam. In most conditions, nucleosomes form orthorhombic crystals. The understanding of the patterns is still under progress.

2- In the second part of this project, we wanted to precisely determine the role played by the histone tails in the supramolecular organization of the dense phases. Recombinant NCPs have been prepared either with all their tails or with their H3-H4 tails deleted. Limited amounts of recombinant NCP can be prepared (tens of milligrams compared to grams of native NCP). To bypass this difficulty, a new method was developed to obtain and analyze microvolumes of NCP dense phases. Only 1 µl of the NCP solution (100mg/ml) is introduced in a glass microcapillary 100 µm thick, followed by the adequate volume of PEG, as usual, and left to stabilize.

We first validated this new method using native NCP. We checked that the phases obtained with this new protocol give rise to the same dense phases previously prepared in standard condition (quartz capillary of 1.5 mm diameter). In particular, the lamello-columnar phase observed in low salt conditions, the 2D hexagonal phase and the 3D orthorhombic phases, present for higher salt concentrations, were found. Moreover, these flat capillaries allow us to control the textures of the phases in polarizing microscopy before and after the Xray experiments.

To improve the stability of histones, we usually add 1 mM of EDTA to all the buffers. In this new series of experiment, we decided to prepare some samples without EDTA. Surprisingly, different organizations were found depending on whether EDTA was present or not. Too few capillaries were prepared and this point has to be clarified with the acquisition of more data.

The other experiments were performed with recombinant NCP. The main results are:

i) For recombinant NCP with all their tails, the 2D hexagonal and the 3D orthorhombic phases were found. Native NCP display a lamello columnar phase for a monovalent salt concentration of 20 mM, we found that recombinant NCP display a 3D orthorhombic organization instead.

ii) In the absence of H3-H4 tails, we found that NCP still form dense phases. The hexagonal or orthorhombic phases were observed at 20 mM and 150 mM salt for PEG 23% and 25% (display on figure 2). We did not explore the whole salt concentration.

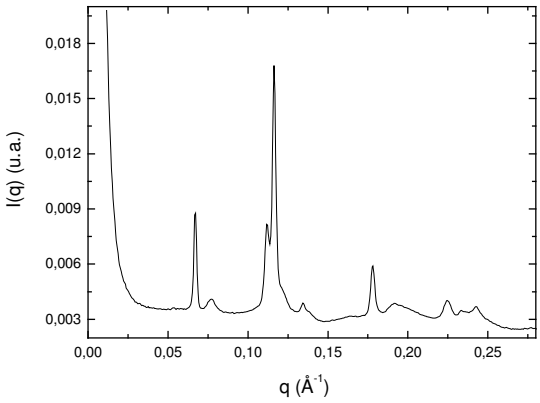

Figure 2: Diffraction pattern recorded for recombinant NCP deleted of their H3-H4 tails, for a monovalent salt concentration of 150 mM and PEG 25%.

For this first series of experiments, we prepared 10 capillaries of recombinant NCP. Surprisingly, we did not record any lamello-columnar phase either for intact NCPs or those with their H3-H4 tails deleted. We would need more data to completely elucidate this point. It would be also necessary to explore the whole range of salt and osmotic pressures to map all the possible interactions of recombinant NCP.

As a conclusion, more than 250 capillaries have been analyzed. All of them have been scanned to find the best X-ray patterns, giving rise to more than 10000 diffractograms. This work was only possible with the high flux of the ID02 line. The phase diagram of NCP in the presence of magnesium and spermidine is almost completed and an article is in preparation. We also demonstrated the feasibility of the experiments with recombinant NCP. This work needs to be continued to completely understand all the effects of the histone tails.