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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

Reports supporting requests for additional beam time

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.

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Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
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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:** DNA condensation in hydrodynamic focusing devices	**Experiment number:** SC-1303
Beamline: ID10B	**Date of experiment:** from: 05.11.2003 to: 11.11.2003	**Date of report:** 12.03.2004
Shifts: 18	**Local contact(s):** Dr. Bernd Struth	*Received at ESRF:*

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Report:

The study of the interaction of biomacromolecules with charged counter ions is of great interest for the understanding of principal biophysical processes and for the development of new materials. Biomolecules like DNA are often found in condensed states e.g. in chromosomes or virus heads. DNA aggregates are also discussed for possible gene carriers in gene therapy. To gain information about these systems a detailed analysis of the aggregation mechanism is needed.

Our model system consists of semidilute DNA solutions mixed with polypropyleneimine dendrimers generation 4 (G4) in aqueous solutions. The dendrimers are spherical polyamine molecules carrying positive charges. The mixing is performed inside a microfluidic mixing device composed of two perpendicular crossing channels (fig. 1). The flows of the biomaterial solutions are controlled by syringe pumps.[1] The syringe pumps are home-build devices which allow us to continuously pump very low rates (10^{-9} l/s). The resulting experimental flow velocities are in between ~100µm/s and 1000µm/s. The channels were spark-erroded into a stainless steel plate (300µm thickness), covered with thin kapton foils and connected to the syringe pumps at the three inlet channels. The system was mounted onto the ID10b goniometer and the x-ray beam was microfocused at the sample position by beryllium compound refractive lenses to a horizontal beam diameter of ~20µm. The scattering image was taken by using a CCD-camera at a sample-CCD distance of 25cm.

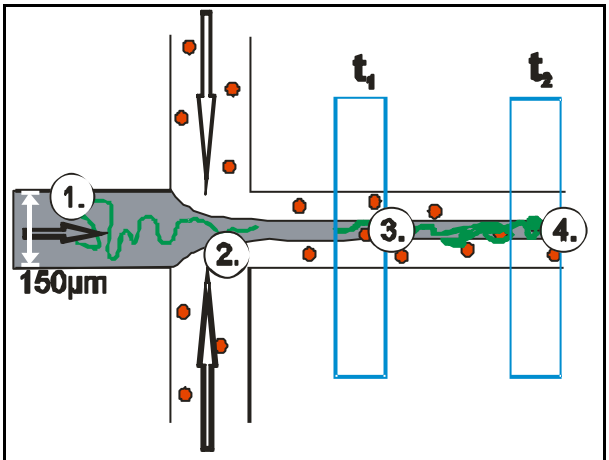

Figure 1:
Sketch of a microfluidic focusing device. The numbers represent the different observation positions discussed in the text.

Using the system sketched in figure 1, flow induced orientation (1), diffusive mixing (2) and time evolution of the reaction (3) & (4) can be studied. A center stream containing long chain molecules gets accelerated and focused by two symmetric side streams. The hydrodynamic focusing area (2), where incoming material (1) senses the maximum elongational flow, gives access to different concentration gradients along the fluid microjet and perpendicular to it depending on the diffusive mixing behavior, the velocity ratio of the center channel to the side channels and the overall flow velocity. For the diffusive mixing of DNA molecules and dendrimers the diffusion of long chain DNA into the G4 solutions can be neglected since the mobility of the long chain DNA molecules is much less than of G4 and additionally the DNA is elongated perpendicular to the diffusion direction. The final concentration at position (4) is known from the initial concentrations and a good approximation for the concentration gradients at positions along the microjet can be achieved by numerical simulations. Since the whole system is dominated by laminar flow each position along the jet represents a steady state in reaction evolution (3) & (4). By tuning the flow velocity we can also tune the time frame which we are looking at. Imaging the area of interest in a step by step scan (cross section of the jet or along the symmetry axis) we can visualize the reaction in a very detailed way.

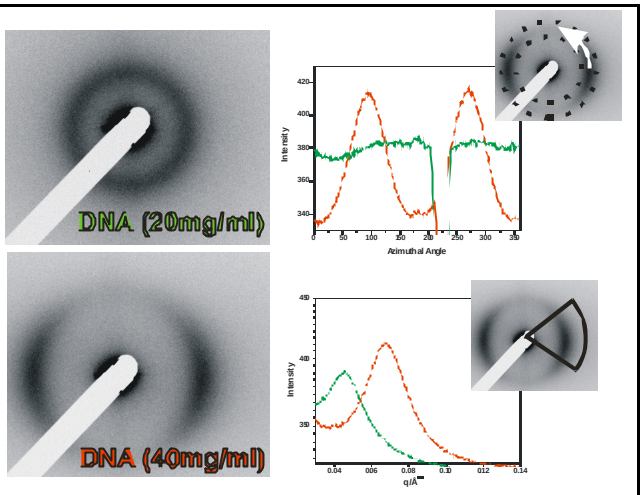

Figure 2:
DNA orientation inside the inlet channel. A DNA solution at 40mg/ml (red) shows a high degree of orientation and a smaller spacing (9.2nm) than an almost unoriented 20mg/ml solution (green, 13.7nm).

In first experiments the hydrodynamic focusing of a center-stream of semidilute DNA solution (polydisperse DNA with an average length of 5µm) by two equivalent side streams of water was observed. The center stream gets accelerated to a fluid microjet and the DNA molecules sense an elongational flow which results in a higher degree of orientation.[2] An additional effect is the pre-elongation inside the inlet channel due to viscous flow. This orientation strongly depends on the concentration of the semidilute DNA solution since the viscosity changes dramatically when going from 20mg/ml DNA to 40mg/ml DNA (fig.2).

In our recent experiments the side streams were additionally carrying a variable amount of G4 dendrimers which are known to condense DNA and generate different collumnar phases depending on the relative charge ratio.[3] The used DNA solution of 10mg/ml does not show a pre-orientation in the inlet channel. Due to the laminar flow inside the microchannel system we can determine the different aggregation states at different positions downward the main channel. Since the dendrimers diffuse inside the DNA jet and a gradient in concentration forms parallel and perpendicular to the microjet, different concentration ratios of DNA to G4 can be found along the fluid microjet. With a focused horizontal beam size of ~20µm we receive a good spatial scanning resolution to resolve different aggregation phases at different positions (fig.3).

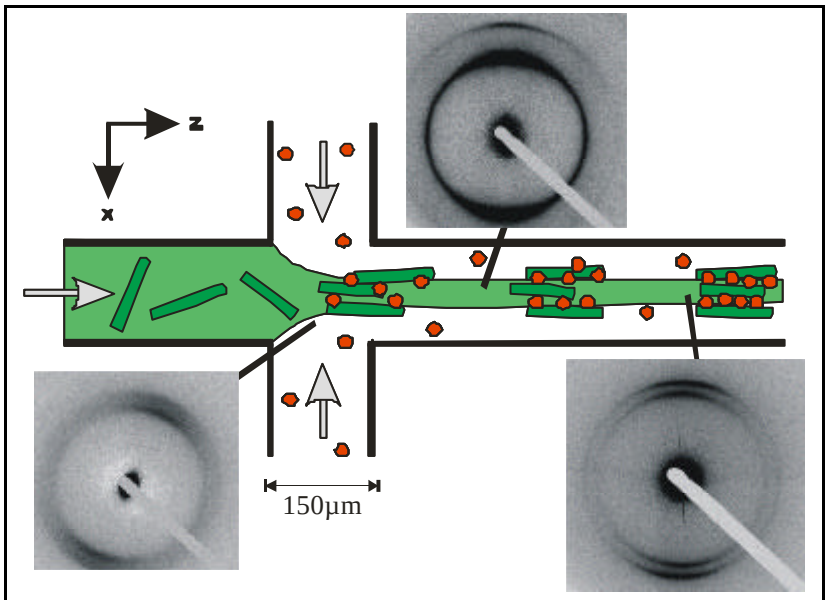

Figure 3:
SAXS images taken at different channel positions. A DNA flow from the center channel mixing with dilute dendrimer solutions from the side channels. Depending on the z-position along the channel, different columnar phases are observed.

Utilizing this method we are now able to measure the whole phase diagram of the different DNA-G4 charge ratios [3] by varying

the observation position and the flow rates. Fig. 4 shows as an example a scan along the z-axis. Here, a transition from the hexagonal (H') to the square regime according to the phase diagram of ref. [3] occurs. In contrast to bulk measurements, our method gives access to investigate the interactions of DNA and G4 in a situation where the DNA/G4 aggregate is elongated due to the hydrodynamic focusing flow.

Two coexisting states of different intensities inside the bulk H' phase can be observed by scanning the imaging position in x-direction from the center of the channel crossing to the microjet boundary with a step size of 20µm (fig.5). In the center region (fig.2-(2)), where the dendrimer concentration is relatively low but the flow elongation is maximal, a double peak referring to a G4 enriched phase corresponding to q_1 and a G4 depleted phase corresponding to q_2 is found. In this state dendrimers are continuously added to the DNA matrix. Regions of an enriched phase in coexistence with regions of a depleted phase including a diffuse transition region in between can be clearly determined (fig. 5b). This can be seen by the transition from the two peaks at d=3.2nm and d=2.6nm to a single peak at d=3.2nm varying the x-position. The depleted phase corresponds to a dense packing of the DNA with an intramolecular spacing close to the diameter of single DNA molecules.

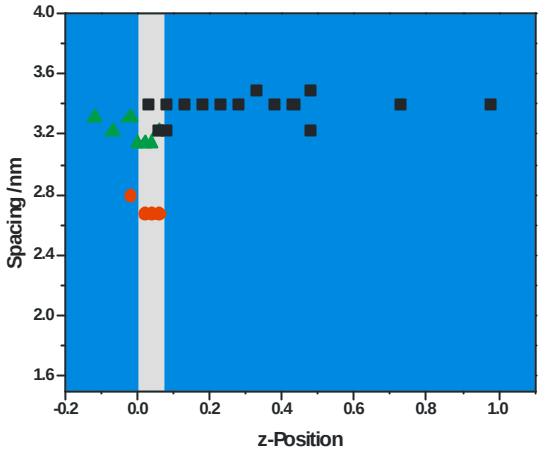

Figure 4:
Spacing along the z-position in the center of the microjet. The grey area represents the transition region from hexagonal' phase to a square lattice phase. A double phase with an dendrimer enriched and a dendrimer depleted state to a square lattice phase can be found in the transition region.

[1] B.Struth, A.Snigirev, O.Konovalov, A.Otten, R.Gauggel, T.Pfohl, Application of microfocusing at a none specific beamline, SRI 2003 proceedings, accepted, **2003**

[2] B.Struth, A.Snigirev, O.Konovalov, A.Otten, T.Pfohl, Confined Biomaterials in Microfluidic Devices, ESRF Newsletter No. 38, Dec**. 2003**

[3] H.M.Evans, A.Ahmad, K.Ewert, T.Pfohl, A.Martin-Herranz, R.F.Bruinsma, C.R.Safinya, Structural Polymorphism of DNA-dendrimer complexes, Phys. Rev. Let., 91, 75501, **2003**

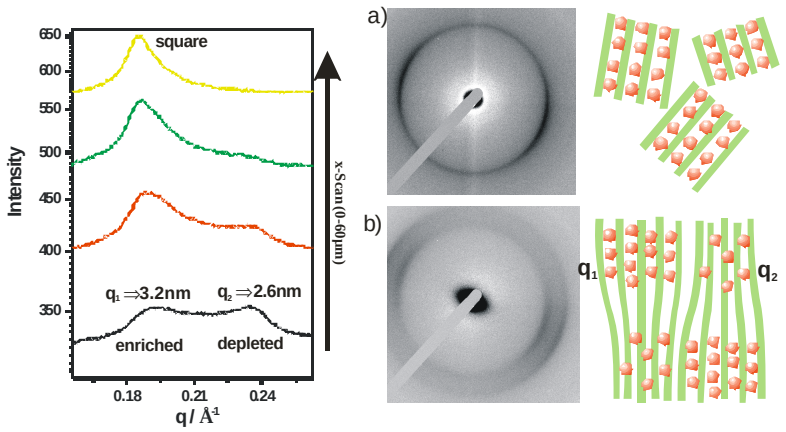

Figure 5:
X-scan from the center of the channel crossing to one boundary of the microjet. The phase coexistence of an enriched G4 phase and a depleted phase in the microjet center (bottom part) according to a continuous integration of dendrimers can be observed. The transition to a square lattice at the microjet boundary is seen in the scattering plots.