The compaction of DNA in cells is designed in a way that a decompaction – for example for transcription and replication processes – is enabled. Both compaction and decompaction results from a combination of "active" processes driven by energy consumption and processes due to electrostatic and electrodynamic interactions. The comparison of DNA compaction by histone proteins (ca. 10 nm diameter, ca. 200 positive surface charges) with dendrimers which act as model histones can help to understand to what extent the consecutive hierarchical organization of chromatin including the wrapping of the DNA around histones happens due to energy consuming processes and how far it is dominated by pure electrostatic and electrodynamic interactions of negatively charged DNA with positively charged macroions. Furthermore, dendrimer/DNA systems are discussed for drug delivery and can be used as model system for supramolecular aggregation.

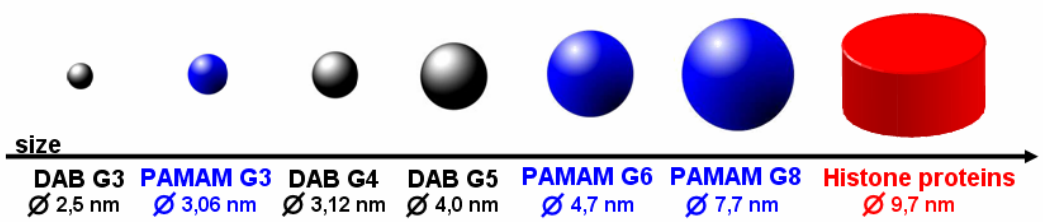

Figure 1: Used dendrimers compared in size to histone proteins.

For understanding and simulating the hierarchical organization of chromatin, we investigate the interactions of aqueous calf thymus DNA-solutions with model histones of increasing size and histone proteins under continuous laminar flow. Using a hydrodynamic focusing microchannel device, a purely diffusive mixing of preorientated DNA molecules with dendrimers is facilitated.[1] Introducing adjustable confinement to the liquid crystalline phases formed by methods of microfluidics enables confinement dimensions comparable to cellular conditions. The small dimensions of the microfluidic devices allow for performing measurements using very low amounts of material.[2]

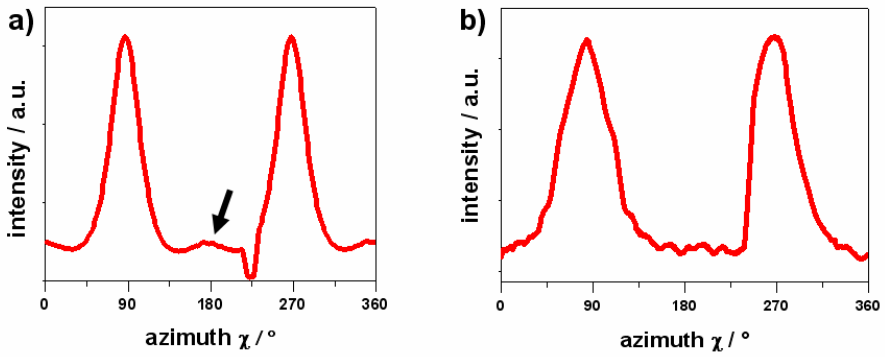

Figure 2: Azimuthal distribution of the scattering intensity of the quadratic phase of G4/DNA for q_{sq} = *0.188 Å*$^{-1}$ (a) and of the quadratic phase of P3/DNA for q_{sq}^{*} = *0.203 Å*$^{-1}$ (b)

As a first histone model system poly-(propyleneimine) DAB dendrimers generation 4 (G4) were used and their interaction with DNA was studied showing the formation of complexes with different lattice structures.[1] By using microfluidic cross channels the

complete mixing phase diagram was determined and a new intermediate phase was found which could not be observed in bulk measurements.[3] In a small region of the quadratic phase of the G4/DNA complexes the azimuthal distribution of the strong scattering peak at $q_{sq} = 0.188\ Å^{-1}$ shows besides strong distribution peaks at 90° and 270°, which correspondent to an orientation parallel to the flow direction, weaker distribution peaks, which are shifted by 90° and therefore indicating an alignment perpendicular to the flow direction (figure 2a). Having the same lattice constant as the stronger peaks one could assume – despite their orientation – that they originate from G4/DNA complexes oriented perpendicular to the microchannel. Contrariwise it may be possible that these weak peaks result from the counter ion lattice of G4. Additional evidence is given by the fact that the lattice constant $d_{sq} = 3.34\ nm$ matches the distance between the helical pitches of B-form DNA[4] indicating a preferential interaction of the polyamine dendrimers with major groove sites of the DNA, which is already known for other polyamines.[5]

For identifying the origin of the additional peaks of the distribution new measurements under identical experimental conditions using poly-(amidoamine) PAMAM dendrimers generation 3 (P3) are performed. P3 are slightly smaller then G4 ($d_{DAB\ G4} = 3.12\ nm$, $d_{P3} = 3.06\ nm$) but still form a quadratic phase. Due to the changed size of P3 a slightly different spacing $q_{sq}^{*} = 0.203\ Å^{-1}$ is detectable showing an orientation of the complexes along the outlet channel. However, there is no evidence for a 90° shifted peak in the azimuthal distribution as observed for G4/DNA (figure 2b). This indicates that the weak distribution peaks in the G4/DNA quadratic phase are not due to complexes with an orientation perpendicular to the outlet channel but can more likely originate in the dendrimeric counter ion lattice.

To better mimic histone proteins dendrimers of increasing size are used. In figure 3a) and b) first measurements of the scattering data from P6/DNA-complexes are shown. With increasing size of the dendrimers the quadratic phase disappears leaving hexagonal phases to be found. Additionally, a transition from hexagonal to lamellar structure is observable indicating a loss of interlayer interaction. This could potentially be a first step towards wrapping of DNA around the dendrimeric model histones of sufficient size. Parallel to testing model histones first measurements of the interaction of histone octamers and histone proteins H1 with DNA were accomplished (figure 3c). A more detailed analysis is in progress.

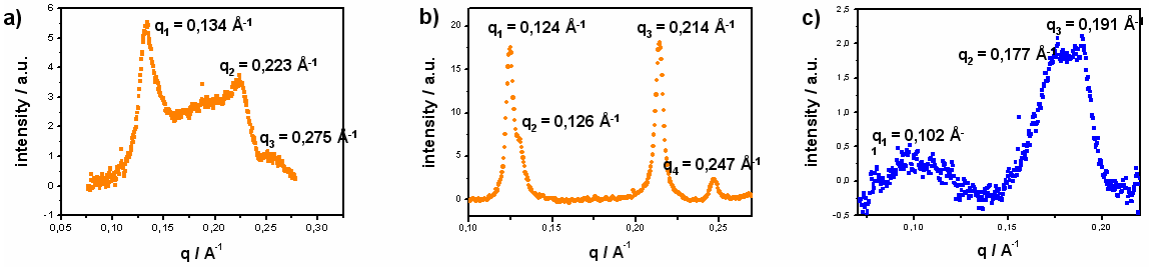

Figure 3: (a), (b) Measurements of P6/DNA complexes, a coexistence of different phases is observable. (c) Scattering data of H1/DNA complexes.

The use of bigger dendrimers and histone proteins leads to reactions on longer timescales due to slower diffusion. Additionally, for forming hierarchical structures which correspond to the chromatin a stepwise adding of different components is needed. Out of

these reasons the ability of adapting the microfluidic devices according to the needs of each analyzed system is of special importance. Due to their inflexibility the spark-eroded channels in steel plates, which we used so far, become therefore unsuitable. For having the possibility to easily adjust its geometry, we started fabricating microfluidic channel systems out of PDMS rubber covered on the top and bottom with kapton or mylar foils using soft lithography techniques. Due to the fact that PDMS rubber disturbs the scattering signal it is of crucial importance to remove the PDMS rubber from the optical path which leads to problems in the manufacturing process.

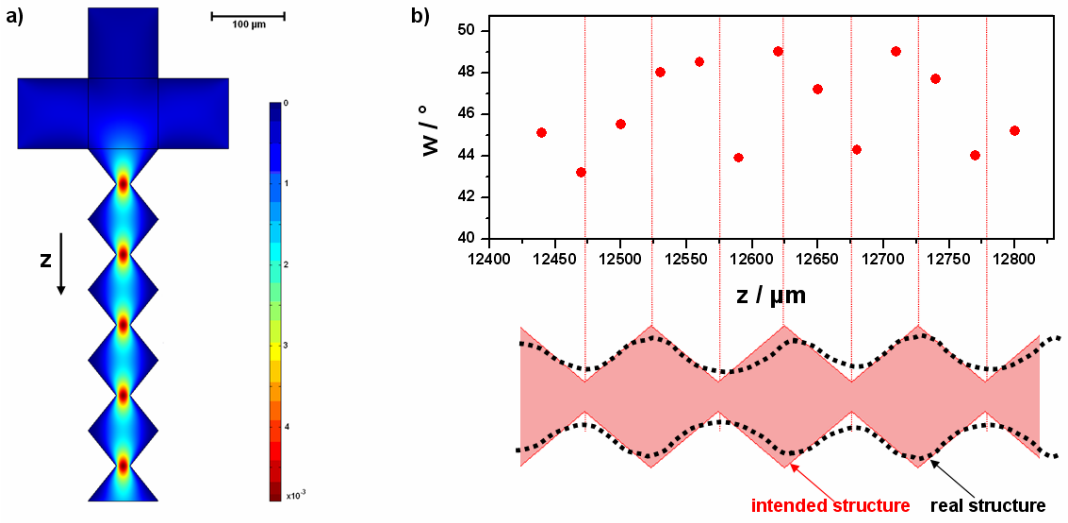

Figure 4: (a) Sketch of the used channel geometry; the velocity profile is simulated by using the program FEMLAB (b) Changes in the orientation of liquid crystal along the outlet channel monitor by the dependence of the FWHM *w*. The discrepancies between real and intended structure are sketched.

A newly designed PDMS device with zig-zag geometry was used for continuous flow of the liquid crystal 8CB (figure 4a). The acceleration at the bottle neck positions is increased leading to a higher degree of orientation followed by relaxation due to a decrease in acceleration in between two bottle neck positions. The resulting oscillation in the degree of orientation is displayed in figure 4b by the changes in the FWHM *w* of the Gaussian fit of the azimuthal distribution at $q = 0.198\ Å^{-1}$ in dependence of the observation position along the outlet channel. The real channel geometry deviates to the intended shape (figure 4b). With minor improvements in the lithographical procedure which is accomplished in house it should be possible to ameliorate the channel structure. These first measurements prove that our new microfluidic devices assembled only of PDMS and kapton/mylar are suitable for X-ray scattering experiments and enable complex geometries which can be adjusted to the experimental needs.

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