SAXS

We confirmed experimentally that DNA scattering peak shifts with DNA concentration as $q^* \sim c^{1/2}$ (Fig. 1). The fit to our data is used as a calibration relationship. That is, we use this relationship to infer effective DNA concentrations from the position of the peak observed for DNA/HA mixtures. The DNA solutions were prepared by iterative dilutions of a mother solution of 200g/L prepared in a polyethylene bag and applied to the sample holder of the SAXS machine. Consecutive dilutions in the bag allow for thorough mixing and for the control of the concentration of solution by weighing.

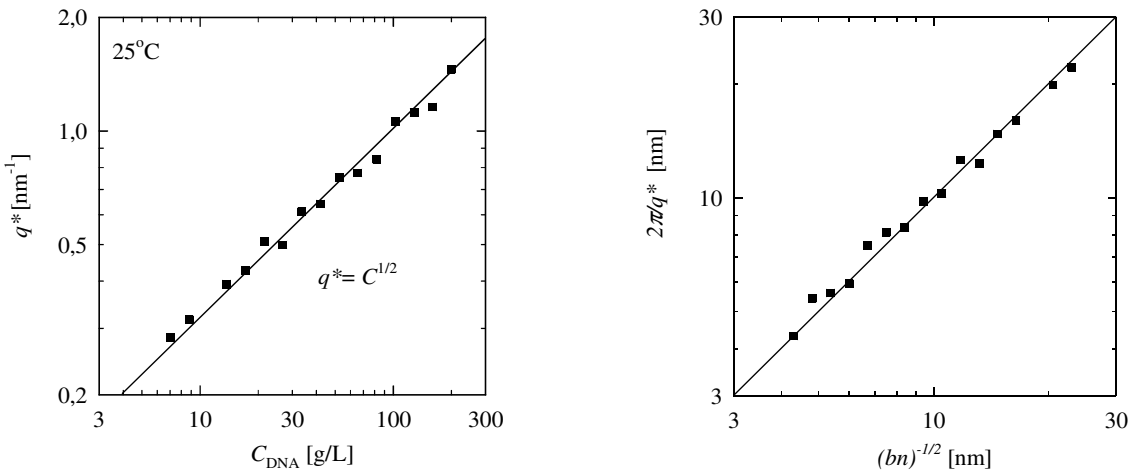

Fig. 1 a) Position of the polyelectrolyte correlation peak, observed in SAXS scattering intensity, shifts with DNA concentration as q*~c^1/2. b) It is evident that the DNA mesh size (bn)^-1/2 is exactly equal to the characteristic length scale 2π/q*obtained by SAXS (DNA monomer length b=0.34 nm, n is the number concentration of DNA monomers)

Using the above, the scattering peaks observed for DNA/HA mixtures may be directly related to the effective DNA concentrations in DNA subdomains. Interestingly, the peak widths (FWHM) are the same for a pure DNA solution and the DNA/HA mixture which contains numerous DNA subdomains of similar effective concentration. In other words, DNA in subdomains is equilibrated and features the same local concentration and the characteristic length scale throughout the sample.

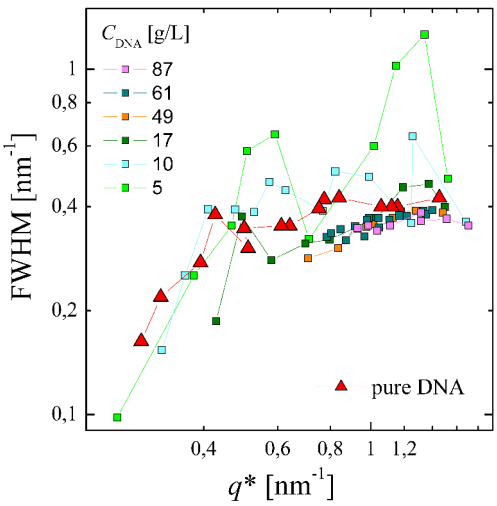

Fig. 2 Peak widths (FWHM) for the pure DNA solutions (from Fig.1 here) and for the DNA/HA mixtures (listed in Fig.1, main article)

Polarizing Microscopy

We attempted to estimate the respective volume fractions of subphases and correlate them with DNA/HA content. In the Fig.3 the PLM images and their analysis are given for DNA 40g/L with HA content 18g/L (1st column), 42g/L (2nd column) and 87g/L(3rd column).Increase in the fraction of nonbirefringent, presumably HA only domains is apparent with the increase in HA. The fraction of the image which is strongly birefringent was taken as the fraction of the volume occupied by the birefringent DNA phase. With l-plate inserted DNA appears blue or orange tinted and the HA should be magenta (red, dark in the images). Without the l-plate, birefringence is identified as bright areas and isotropic HA domains appear black. Images are analysed with appropriate color thresholds and white is superimposed to highly birefringent and blue to isotropic regions. Surfaces of blue and white regions are then integrated and identified as fractions of the two phases.

We present images both with and without lambda plate inserted. Image analysis of both types of images was mutually adjusted to give consistently similar fractions of DNA or HA domains – i.e. blue and white domains in 2nd and 4th row appear similar and similar DNA/HA volume fractions are thus extracted from these images.

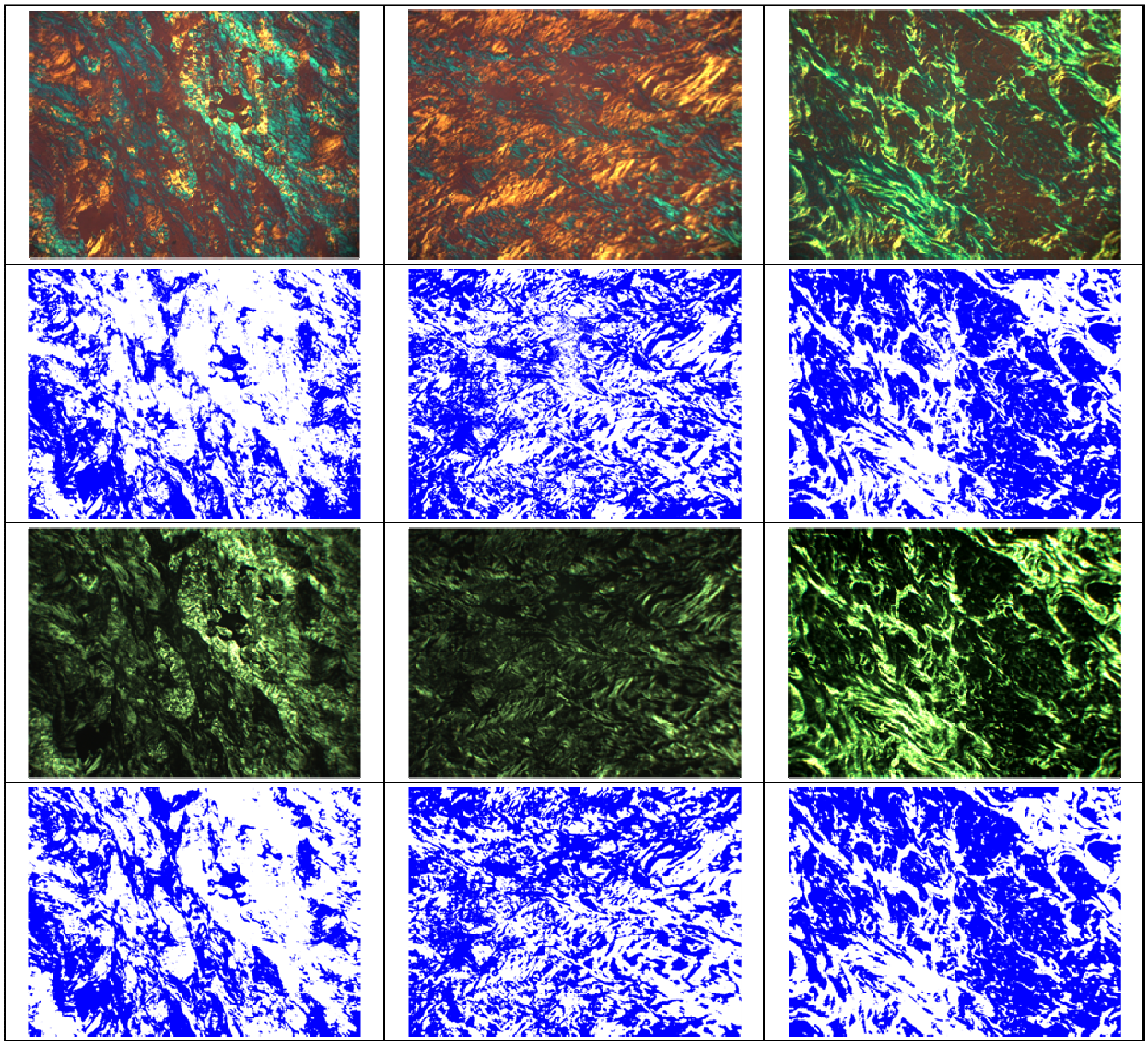

Fig.3. Polarizing microscopy (PM) images of DNA 40 g/L + HA mixtures. First column HA 18g/L, second 42g/L, third 87g/L.. First row: PM images with \lambda-plate (full wave plate) inserted. Nonbirefringent areas appear dark red while birefringent areas appear blue or orange. Negatively birefringent DNA molecules appear orange when parallel to the \lambda-plate fast axis (arrow). Third row: Same areas imaged without \lambda-plate, only with crossed polars..

Second and fourth row represent image colour threshold analysis where blue is ascribed to nonbirefringent and white to birefringent areas. Two PM image types are analysed for comparison and calibration of the color thresholds. Scale bar 1mm.
Eventually we compare these fractions with those obatined from SAXS determination of DNA, HA concetrations and respective volumes. The two sets do not match, there is only agreement in the sense that adding more HA does lead to more HA domains to be bserved by both methods.
Presumably, as PLM slide and coverslip preparations are not thin enough there may be overlap between HA and DNA domains in z-direction. Thus the border between the domains is not well defined and the PLM analysis can not provide absolute fractions – as these numbers depend on the threshold color defined to distinguish between the domains.

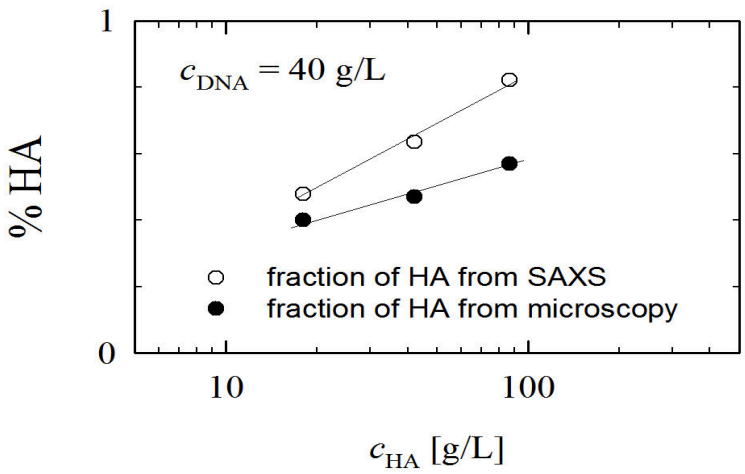

Fig.4. Comparison of volume fractions of DNA and HA subdomains estimated from SAXS and from the above presented analysis of PLM images. The absolute values are not similar, however, there is a qualitative agreement.