

CONFORMATIONAL DYNAMICS OF IMMOBILIZED DNA IN EXTERNAL FIELDSJ.Koota, R.Lehner, G.Maret & [T.Gisler](#)*Universität Konstanz, Fachbereich Physik, Fach M621, 78457 Konstanz, Germany.*

Polyelectrolytes grafted to surfaces are ubiquitous in biology where they are important for cellular recognition. In addition, they are beginning to play an increasingly important role for responsive materials or for biocompatible surfaces.

Despite considerable theoretical and simulation work on surface-grafted polyelectrolytes, experimental data are still scarce, mainly due to the lack of suitable systems with well-defined chain length and homogeneous distribution of charged sites.

These problems plaguing the use of synthetic polyelectrolytes are however largely circumvented when DNA is used. Its large size (λ -DNA has a contour length $L=16\mu\text{m}$ and a radius of gyration $R_g=0.8\mu\text{m}$) allows visualization by fluorescence microscopy, and the conformational dynamics is sufficiently slow that it may be followed by direct imaging using CCD cameras.

In this contribution we report on the preparation of carpets of end-functionalized λ -DNA and their characterization by confocal fluorescence microscopy (CFM).

We have developed a robust protocol for streptavidin-functionalization of common float glass, mica, as well as indium-tin oxide (ITO) and zinc oxide, to which the biotin-functionalized DNA selectively attaches over a large range of pH values. We find that obtaining a dense and homogeneous silane layer on the primary substrate material is the bottleneck for efficient attachment of DNA to the substrate.

The density of DNA attainable is, however, limited by the excluded volume of the DNA. Simple deposition of end-functionalized DNA results in densities slightly smaller than $1/(\pi R_g^2)$. Higher densities for dilute supernatants were obtained by pushing DNA to the surface by an electric field perpendicular to the surface, using conducting coatings such as ITO or ZnO, and a Pt counterelectrode. ZnO, however, was found to be unstable in common DNA buffers containing EDTA due to the complexation of Zn^{2+} ions by the EDTA.

For confocal fluorescence microscopy (CFM) we have labelled the DNA with the intercalation dye YOYO-1 at an average dye concentration of 1 molecule per 5 base pairs.

Applying the electric field perpendicular to the surface and parallel to the optical axis of the microscope then resulted in an extension of the coiled DNA molecules, allowing to distinguish individual DNA molecules (see Fig. 1).

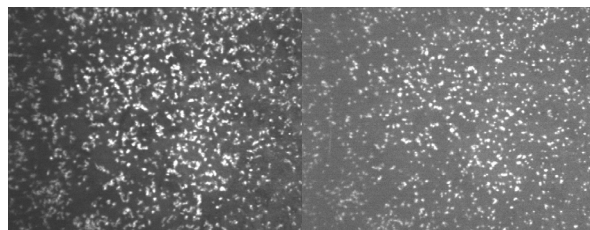


Fig. 1: Confocal fluorescence microscopy images of YOYO-1 labelled λ -DNA grafted to an ITO coated glass slide. Left: relaxed, coiled DNA in the absence of an electric field. Right: the DNA molecules are extended by the electric field. Their cross-sectional area observed by CFM is reduced due to the restricted excursions of the DNA chain transverse to the field direction. Field of view: $70\mu\text{m} \times 90\mu\text{m}$.

We have also been able to obtain two-sided attachment of λ -DNA which was functionalized with biotin at one end and with a thiol group at the other end. This procedure then allowed to extend the DNA directly using piezo-driven mechanical cantilevers. This method has the advantage over electric field stretching that no currents are required which lead to faster bleaching of the fluorescent marker.

We discuss our results in view of recent theories for the conformation of isolated polyelectrolyte chains extended by electric fields.

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