

DNA-INTERCALATING SURFACE COATING

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INTRODUCTION: A variety of noncovalent coupling techniques are known to bind DNA onto glass.[1] In this study we report a novel efficient method for unspecific binding of double stranded DNA (dsDNA) onto coverslips. This approach is based on the strong affinity between dsDNA and a modified intercalator covalently bound to 3-Aminopropyltrimethoxysilane and 1,4-Phenylenediisothiocyanate coated glass surface (Fig. 1). The intercalation of a Cy5 labelled, doublestranded DNA-fragment (2 kilobase pairs (kbp)) on the surface can be observed by supercritical angle fluorescence (SAF) measurements with a custom-made biosensor [3]. Briefly, after excitation by a HeNe laser a parabolic glass lens collects only the fluorescence emitted into the angular region above the critical angle of refraction, which corresponds to $\sim 61^\circ$ for a glass/water interface. By detecting only supercritical emission, the detection volume is restricted to a surface distance well below 100 nm, while most of bulk fluorescence is rejected [4].

METHODS: The synthesis of the amine modified pyrene (1) is described in [2]. After fixation of the intercalator on coated coverslips (Genorama SAL, Asper Biotech, Estonia), the surface was treated with $\text{CF}_3\text{COOH}/\text{CH}_2\text{Cl}_2$ (1:1) for 3 h to remove the BOC protecting groups (Fig. 1). Completion of the coupling was checked by absorption spectra.

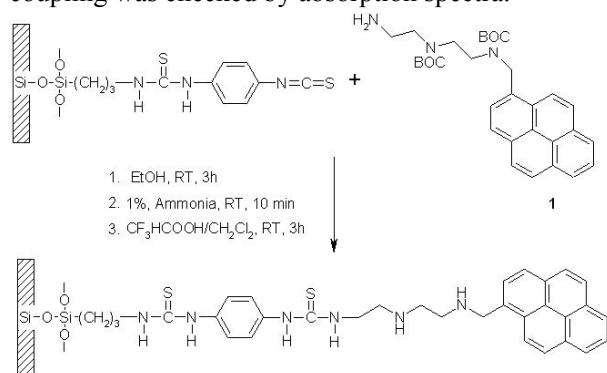


Fig. 1: Reaction scheme for the coupling of the the modified pyrene to 3-Aminopropyltrimethoxysilane +1,4-Phenylenediisothiocyanate coated coverslip.

Subsequently the coverslips were glued onto a measuring cell containing six reaction chambers. For SAF-measurements a solution containing 2 kbp dsDNA-fragments ($150\mu\text{L}$, 10^{-10} M, TE-buffer, pH

7.1) was pipetted into the chamber. Each fragment was labelled by one Cy5 dye molecule.

RESULTS: As reference for the SAF measurements a SAL slide was prepared without intercalator attached to the surface. The addition of the dye-labelled DNA to this surface caused a rapid increase of the fluorescence to 16 ± 2 kHz, which remained at this level afterwards (Fig. 2). This count rate can be attributed to non-specific interaction of the DNA-fragment with the surface. In contrast the use of DNA-intercalating surface-coating led to a fluorescence increase of more than 80 ± 7 kHz (Fig. 2). Comparisons with FRAP (fluorescence recovery after photobleaching) experiments also revealed this effect and indicated that the noncovalent coupling is caused by intercalation.

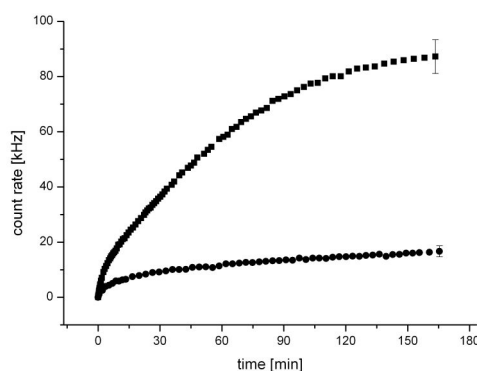


Fig. 2: SAF intensity after addition of Cy5-dsDNA to DNA-intercalating (■) and non intercalating surface (●). The background was subtracted from the data and the start time was set to zero.

DISCUSSION & CONCLUSIONS: Our results stringently demonstrate the binding of the DNA to the surface induced by the immobilized intercalator. Thus, the binding efficiency of dsDNA to surfaces is improved by coupling of an intercalator to the substrate. This method could be an application in different types of DNA analysis.

REFERENCES: ¹N. Zammateo, L. Jeanmart, S. Hamels, et al. (2000), *Anal. Biochem.* **280**, 143. ²P. Häfliger, R. Alberto, (2003) *in preparation*. ³T. Ruckstuhl, M. Rankl and S. Seeger, (2003) *Biosens. Bioelectron.* **18**, 1193. ⁴J. Enderlein, T. Ruckstuhl and S. Seeger (1999) *Appl. Opt.* **38**, 724.