

SPECIFICITY OF DNA HYBRIDISATION ON A FUNCTIONALISED LIPID BILAYER

INTRODUCTION

We have investigated the possibility of using the QCM-D technique and surface immobilized synthetic peptide nucleic acid (PNA) and DNA, as selective probes for DNA (Höök F et al.). Streptavidin was immobilized on a fluid biotin-containing phospholipid bilayer supported on a SiO₂ surface in order to allow oligonucleotide attachment and minimize unspecific binding during subsequent DNA hybridisation.

RESULTS

The initial rapid increase in D followed by a slower decrease during the continuous binding (indicated by a decrease in f) of streptavidin indicates transformation from a non-rigid to a rigid layer of attached proteins (**Figure 1**). This is interpreted as a direct measure of protein-2D-crystal growth, as also supported by, e.g., atomic force microscopy images of similar protein-lipid systems [Reviakine, I., et al. J. Structural Biol, vol. 121 p.356 (1998)].

Mixed-sequence 15-mer biotin-PNA and DNA, respectively, with identical base-pair sequences, were immobilised on top of the streptavidin layer. The samples were subsequently exposed to 1 mM of complementary DNA at 24°C (see **Figure 2** below).

The binding rate of complementary DNA was different to the immobilised PNA and DNA strands, respectively, indicating

a difference in the association rate at 24°C (not shown).

Also various mismatched DNA were investigated. Only the fully complementary and singly mismatched DNA oligomers hybridized with the immobilised PNA and DNA, demonstrating a high selectivity with no influence from unspecific binding. In the case of single mismatch the binding was weaker and reversible compared to the fully complementary strand, which can be seen in **Figure 2**.

It should be noted that the larger ratio between ΔD and Δf (i.e. dissipation per bound mass) for the PNA-DNA compared to the DNA-DNA hybrids (see the ΔD vs Δf plot, **Figure 3**) indicates that the PNA-DNA hybrid has a more flexible and elongated structure, which proves the QCM-D technique capable of

detecting structural differences between various oligonucleotide strands.

CONCLUSIONS

By using an inert background consisting of functionalised lipid bilayer, QCM-D shows remarkable specificity valuable for biomolecular interaction research. During DNA hybridisation, a single mismatch in a 15-mer strand results in less adsorption and reversible instead of irreversible binding, compared to the fully complementary strand.

REFERENCES

Characterisation of PNA and DNA immobilisation and subsequent hybridisation with DNA using acoustic-shear-wave attenuation measurements. Langmuir 2001, 17, p. 8305-8312
 Höök, F, Ray A, Krave U, Norden, B and Kasemo, B

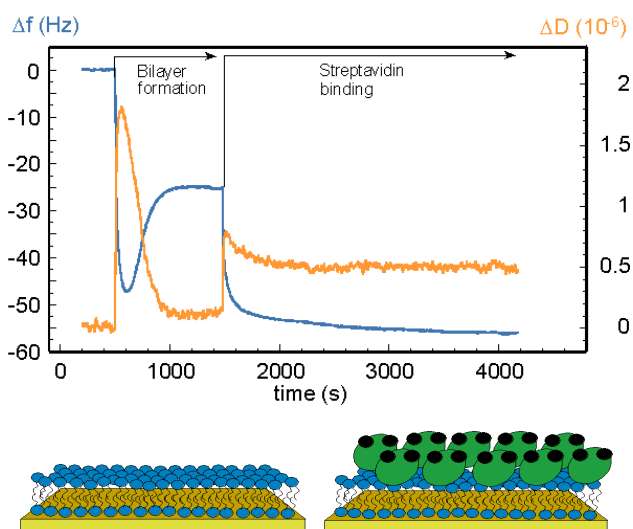


Figure 1 – Formation of a biotinylated lipid bilayer followed by immobilization of a streptavidin protein layer.

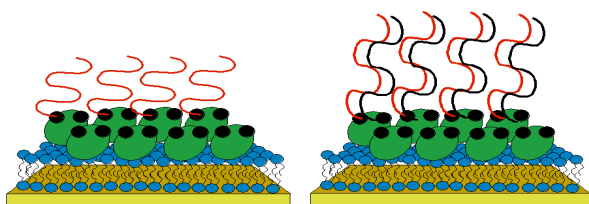
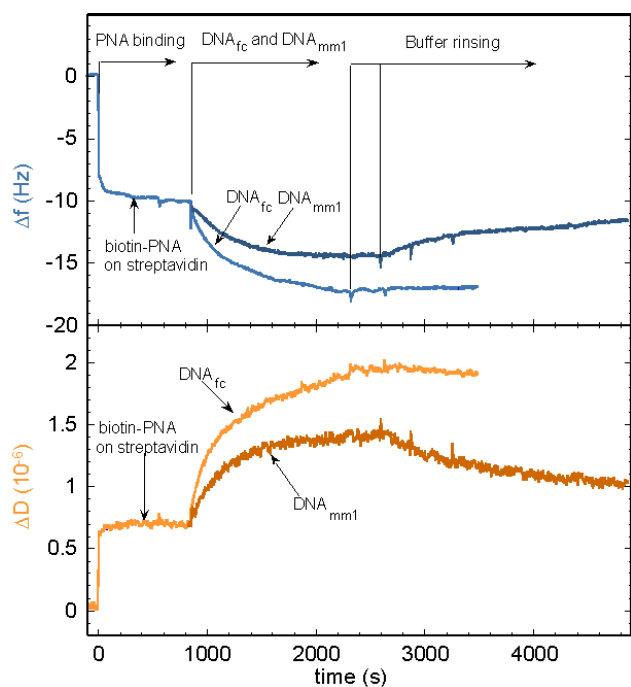


Figure 2 – Binding of single stranded biotinylated PNA to Streptavidin followed by specific hybridization of complementary and single mismatch DNA strands, respectively.

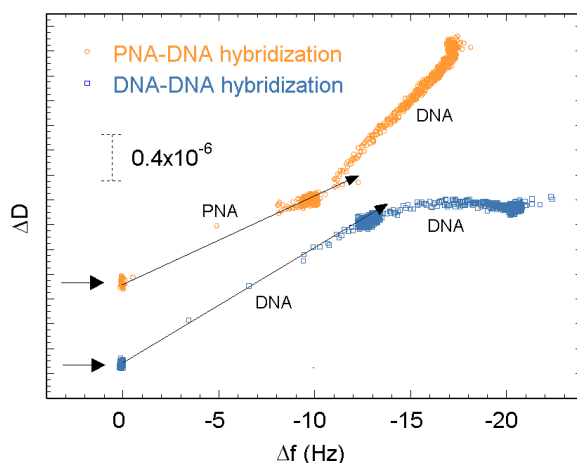


Figure 3 – Structural differences between hybridized single strands of PNA-DNA and DNA-DNA.