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Sequential entrapment of PNA and DNA in lipid bilayers stacks

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Sequential immobilization of single stranded DNA and complementary PNA molecules in thermally evaporated fatty amine films is demonstrated and evidence for their *in-situ* hybridization is presented.

In a pioneering report, Nielsen and co-workers demonstrated that the entire sugar-phosphate backbone in DNA could be replaced by an *N*-(2-aminoethyl)glycine based polyamide structure and that the DNA bases could be attached to the peptide backbone yielding a class of molecules known as peptide nucleic acids (PNA).[1] Since the discovery of this DNA mimic and subsequent demonstration that PNA molecules obey the Watson–Crick base pairing rules[2] and that they bind with higher affinity to complementary oligonucleotides, PNA has emerged as one of the most suitable candidates for DNA diagnostic[3] and therapeutic applications.[4,5] In this communication, we show for the first time that PNA may be immobilized in thermally evaporated lipid films by simple immersion of the lipid film in the PNA solution and furthermore, that this may be extended to a sequential PNA–DNA immobilization strategy for DNA detection.

Oligonucleotides corresponding to the sequence AGTGATCTAC (**1**) and AGTGATCCAC (**2**) were synthesized as described elsewhere.[6] The PNA oligomer H-GTAGATCACT-$NH(CH_2)_2$-COOH was synthesized manually.[7] **1** is complementary to the PNA sequence while **2** is a single mismatch sequence to the PNA sequence. Pre-formed DNA–PNA solutions were obtained by mixing equimolar quantities of **1** and PNA solution under standard hybridization conditions. 250 Å thick octadecylamine (ODA, Aldrich) films were thermally evaporated onto gold-coated 6 MHz AT-cut quartz crystals, and quartz substrates for quartz crystal microgravimetry (QCM) and UV-melting measurements respectively.[8] The thickness of the films was cross-checked using ellipsometry.

UV-melting experiments on the ODA–DNA/PNA conjugate films were carried out as a test of hybridization. Unlike in the case of hybridization of complementary DNA strands where fluorescent intercalators such as ethidium bromide are routinely used,[6] reports on the use of intercalators for the detection of PNA–DNA hybrids are relatively scarce.[9] Fig. 1A shows plots of the UV-melting data recorded from ODA films in which preformed PNA–DNA duplexes were entrapped (triangles); an ODA film with sequential immobilization of **1** and PNA (squares); an ODA film with **2** and PNA intercalated sequentially (circles) and an ODA film first immersed in PNA followed by **1** (diamonds).[10] The specificity of base-pairing in the DNA–PNA complexes within the ODA matrix is reflected in the lowering of T_m in the case of single base pair mismatch DNA (**2**) with PNA which is much lower (49 °C) than the T_m of complementary DNA (**1**) with PNA (59 °C). Introduction of a single base pair mismatch in the DNA sequence leads to destabilization of the DNA–PNA complex with a lowering of the melting transition temperature. An interesting result of the investigation is the fact that the melting transition of DNA (**1**)–PNA hybrids in the lipid matrix (66 °C, squares) is much higher than the T_m of the ODA film in which preformed DNA–PNA duplexes were incorporated (59 °C, triangles). Another important observation is that the order of immobilization of PNA and DNA in the ODA matrix is not of much consequence (T_m of PNA–DNA hybrids formed by first entrapping PNA and then DNA = 68 °C, diamonds) in the extent of hybridization. The increased stability of the PNA–DNA hybrids formed in the ODA bilayers *vis-à-vis* the duplexes formed in solution and then entrapped within the lipid may be due to the following reason. During sequential immobilisation of DNA followed by PNA (or *vice-versa*), electrostatic attachment of DNA to the ODA monolayer would leave free and exposed the bases on the DNA molecules. Fig. 1B shows a schematic of the expected molecular structure of the ODA–DNA monolayer within the bilayer assemblies after entrapment of PNA. This mode of organization would then enable facile hydrogen bonding of the complementary PNA molecules with the immobilized DNA (Fig. 1B), a process not so easily done in solution. It is also possible that entrapment of pre-formed PNA–DNA complexes would lead to some orientational disorder within the ODA matrix and therefore reduced binding with the lipid. This would be reflected in terms of a lowering of the melting transition temperature as observed in this study. The UV-melting measurements clearly demonstrate that PNA–DNA immobilization and hybridization occurs within the confines of ODA bilayer stacks and furthermore, that it is sensitive to single base mismatches in the DNA sequence.

Fig. 2 presents quartz crystal microgravimetry (QCM) data of the diffusion of the DNA and PNA molecules into the fatty amine films during immersion of the ODA-coated QCM crystals in 1 μM aqueous solutions of **1** followed by PNA (triangles); **2** followed by PNA (squares) and preformed PNA–DNA complexes (circles).[11] In all cases, it is observed that there is extremely rapid diffusion of the DNA and PNA molecules into the lipid matrix. While the diffusion of DNA molecules (both **1** and **2**) into the ODA film is not surprising and may be explained in terms of attractive electrostatic interaction between

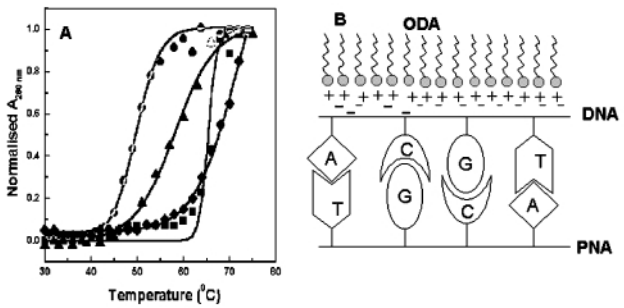

Fig. 1 A) UV melting data for 250 Å thick ODA films on quartz after immersion in 1 μm solutions of **2** followed by PNA (circles); preformed PNA–DNA complexes (triangles); **1** followed by PNA (squares) and PNA followed by **1** (diamonds). The solid lines are sigmoidal fits to the data. B) Schematic showing possible microscopic structure of DNA–ODA composite monolayer binding with complementary PNA.

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the negatively charged DNA phosphate groups and protonated amine groups in the lipid matrix,[12] the significant intake of the extremely weakly charged PNA is clearly due to other interactions. The large PNA uptake is all the more interesting since the presence of DNA molecules **1** and **2** in the ODA matrix should effectively block binding sites with the ODA molecules. It is tempting to attribute the PNA diffusion into the ODA matrix to interactions arising from hybridization of the PNA molecules with the already immobilized DNA. However, a comparison of the QCM curves from the sequential immersion of the ODA films in **1** and **2** followed by immersion in PNA solution (triangles and squares respectively, Fig. 2) reveals that the mass uptake due to PNA alone (the mass increase in the second part of the QCM mass uptake curves) is similar in both cases. This clearly shows that hybridization alone cannot account for the diffusion of PNA into the DNA–ODA films and entropic factors/other interactions drive the diffusion of PNA into the lipid matrix. Indeed, immersion of the ODA films first into PNA solution followed by DNA (data not shown) resulted in significant PNA entrapment further attesting to the above fact. Even though QCM may be used to determine the presence of DNA and PNA molecules in the ODA matrix, it is clearly insufficient in determining whether hybridization had occurred between the entrapped PNA and DNA molecules. Equilibration of the number density of preformed PNA–DNA complexes in the ODA matrix (circles) occurs on time-scales similar to that observed for the single-stranded DNA molecules **1** and **2** as well as PNA. The lower mass uptake in this case (preformed PNA–DNA complexes) may be due to duplex structure preventing complete neutralization of the negative charge on the DNA by the positively charged ODA matrix. Further experiments are required to clarify this point. From the QCM measurements presented in Fig. 2, optimum times of immersion of the ODA films in the different DNA and PNA solutions were determined and used in the preparation of ODA–DNA/PNA composite films for UV melting measurements (Fig. 1A).

To conclude, it has been demonstrated that DNA and PNA molecules may be entrapped in thermally evaporated fatty amine films thus leading to a potentially exciting and novel protocol for the entrapment of oligonucleotides and their analogues. This strategy has been shown to lead to the hybridization of complementary PNA–DNA sequences in the lipid matrix in a sequential immobilization experiment. The specificity of hybridization within the lipid matrix is good and may be easily detected by UV-melting measurements. The possibility of depositing patterned lipid films and entrapping different PNA sequences in the different elements makes this approach promising for gene sequencing by hybridization techniques. A highlight of this approach is that it is both PNA and DNA friendly — no (fairly complicated) thiolation of these molecules needs be done, as is required in other gold film-based immobilization strategies.[3b]

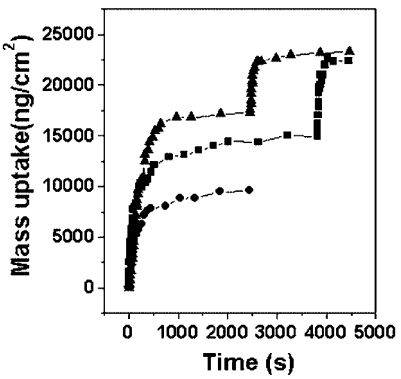

Fig. 2 QCM mass uptake data recorded during immersion of 250 Å thick ODA films in 1 μm solutions of preformed PNA–DNA complexes (circles); sequential immersion in **2** followed by PNA (squares) and **1** followed by PNA (triangles).

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Notes and references

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7 Merrifield resin derivatized with β-alanine (0.013 meq g^{-1}) employing the standard Boc-protection strategy was used in the synthesis. Cleavage from the solid support using the TFA–TFMSA cleavage procedure yielded the PNA oligomer with a free carboxylic acid at its 'C' terminus. The oligomer was purified by RP-FPLC and its purity rechecked by RP-HPLC and confirmed by MALDI-TOF mass spectroscopy.

8 The films were deposited in an Edwards E306A vacuum coating unit operated at a pressure of better than 1×10^{-7} Torr. The thickness of the deposited films was monitored *in-situ* using an Edwards quartz crystal microbalance (*QCM*).

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10 The UV melting experiments were carried out on Perkin Elmer Lambda 15 UV/VIS spectrophotometer fitted with a Julabo water circulator with programmed heating accessory. The quartz substrates bearing the films were cut so as to fit into the cuvette normally used for liquid samples. The DNA–ODA films were heated at a rate of 0.5 °C per minute and the thermal denaturation of the duplex was followed by monitoring changes in the absorbance at 260 nm as a function of temperature. A 250 Å thick ODA film on quartz was taken as the reference.

11 This was achieved by *ex-situ* measurement of the QCM resonance frequency after thorough washing and drying of the crystals using an Edwards FTM5 frequency counter. This frequency counter had a resolution and stability of 1 Hz. The frequency change (Δf) was converted to mass loading (Δm) using the relationship $\Delta m = 12.1\ \Delta f$ (ng cm^{-2}).

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