Oral

DNA MUTATION DETECTION IN AN ON-CHIP EXPERIMENT USING SPR IMAGING: EFFECTS OF TEMPERATURE, SALINITY AND FORMAMIDE ON DNA MELTING

Julia Fuchs, Daniela Dell'Atti, Roberto Calemczuk, Arnaud Buhot, Marco Mascini and Thierry Livache

CREAB, UMR 5819 (CEA, CNRS, UJF), Institut Nanosciences et Cryogénie, Grenoble, France

Arnaud.Buhot@cea.fr

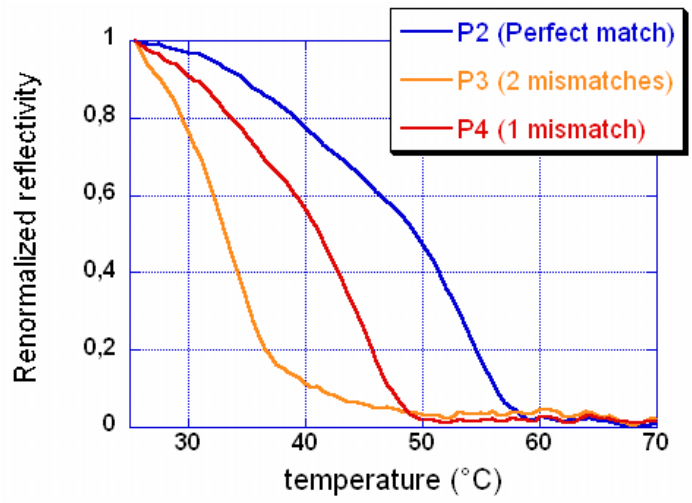
Since DNA hybridization is probably amongst the best understood biointeractions, there is a constant interest in DNA based analysis for all kinds of applications. Some have found their way into clinical diagnostics to analyze gene expression profiles or carcinogenic tissue; others are still subject to ongoing research focussing more and more on complex samples from PCR to genomic DNA. As the technology of DNA microarrays is extending with an uncountable number of chip designs and readout protocols, an increasing need for DNA models taking into account all kind of surface and buffer interactions emerges. Perfect knowledge of DNA hybridization in on-chip experiments is inescapable, especially when it comes to the detection of single nucleotide polymorphisms (SNP), a delicate and challenging task for large scale micro-array analysis.

This work presents a systematic approach to study DNA surface hybridization and thermal denaturation using an original system of Surface Plasmon Resonance imaging (SPRi) coupled to a precise temperature regulation. This unique combination enables real-time detection of DNA binding to the functionalized surface in a temperature dependent manner without any need for target labelling. To immobilize DNA on the gold coated prism, we use an optimized poly-pyrrole electro-polymerization process that proved extremely stable agains heating cycles [1]. Recently we demonstrated the application of this system to point mutation detection [2]. By tracing DNA thermal denaturation, we are able to discriminate between wild type and mutation sequences differing by a single base. Moreover, we showed the interest of such a system by detecting also mixtures containing only small percentages of mutated DNA amongst the wild type DNA. This feature is particularly interesting as it makes the system suitable for various kinds of samples, from blood or tissue cell extracts.

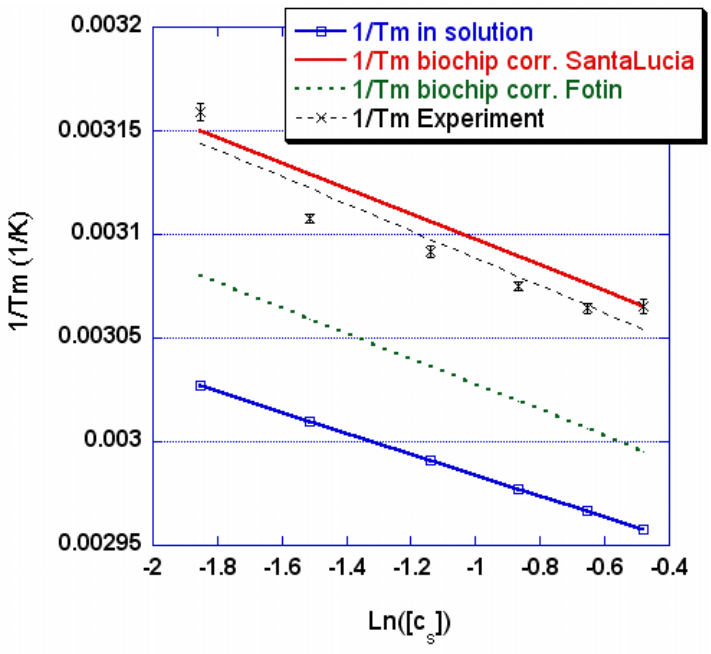
To optimize point mutation detection using thermal denaturation, we carried out a thorough study focused on the influence of currently used buffer components on oligonucleotide melting. In a first step, DNA equilibrium melting curves in PBS buffers of varying ionic strength are acquired which allows to obtain chip specific thermodynamic parameters, i.e. enthalpy and entropy of the DNA hybridization. Although the study is carried out for one set of sequences, it demonstrates the possibility of improving the predictions of the melting temperature by taking into account chip specific corrections. The influence of the buffer's salinity on the hybridization is compared to predictions in solution by the Nearest Neighbour model [3]. In a second step, the buffer was enriched by different percentages of formamide, a DNA denaturing agent currently applied in on-chip analysis. We can show that in average, every percent of formamide reduces the dissociation temperature by 0.57°C. Furthermore, the denaturant effect of formamide can be advantageous to reduce secondary structures in long targets. In addition, formamide has also a positive influence on the noise of the measurement.
We present a label-free technique with exceptional prospects for the establishment of biochip DNA hybridization predictions which can improve chip design and protocols for DNA microarray analysis.

[1] P. Guedon, T. Livache, F. Martin, F. Lesbre, A. Roget, G. Bidan, Y. Levy, **Anal. Chem.**, 72 (2000) 6003
[2] J. B. Fiche, J. Fuchs, A. Buhot, R. Calemczuk, T. Livache, **Anal. Chem.**, 80 (2008) 1049
[3] J. Santa Lucia, Jr, **Proc. Natl. Acad. Sci. USA 95** (1998) 1460

Figures:



DNA thermal denaturation target T2 hybridizing perfectly to probe P2 and presenting on mismatch when hybridized to P4 and 2 adjacent mismatches when hybridized to P3. The denaturation curves are carried out in 10% formamide permitting a low-noise signal and good mismatch discrimination.



Comparison of the influence of the ionic strength of the hybridization buffer on the melting temperature observed on-chip. While the slope follows quite well predictions from DNA experiments in solution, the melting temperatures observed in experiment are not reflected by predictions from the Nearest Neighbour model, either in solution or for biochip methods. This method can thus be used to improve biochip corrections to enhance SNP detection.