

Poster

**SURFACE PLASMON RESONANCE IMAGING WITH TEMPERATURE
REGULATION: A VERSATILE INSTRUMENT FOR DNA BASED
BIOANALYSIS**

Julia FUCHS, Jean-Bernard FICHE, Christelle CORNE, Daniela DELL'ATTI, Roberto CALEMCZUK, Arnaud BUHOT, Didier GASPARUTTO, Alain FAVIER, Marco MASCINI* and Thierry LIVACHE.*

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

**Dipartimento di Chimica, Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino (Fi), Italy*

Arnaud.Buhot@cea.fr

For the last two decades, surface plasmon resonance imaging (SPRi) has known a great success for chemical and, in particular, biological interaction studies (1). It has become widely employed in bioanalysis since this spectroscopy method allows the real-time monitoring of label-free interactions on a metal surface. Working in aqueous environments, the metal of choice is gold, which, being chemically inert, gives way to well-established surface chemistries employing dextran matrices, self-assembling monolayers of thiols or poly-pyrrole electrospotting, for example. The SPRi technique has been proven a versatile tool for biological interaction studies, notably for screening applications and kinetics studies.

This poster will present an original approach to couple the SPRi technique with a precise, local temperature control that enables biological studies in a flexible environment. By controlling the temperature of the sample cell between 20 and 85°C with a precision of 0.05°C (2), we can follow binding and dissociation events and, in this way, extract temperature dependent information. Combined with a poly-pyrrole electro-spotting method, we obtain functionalized DNA chips that can undergo multiple heating cycles without deterioration.

The poster will present results of on-chip DNA melting and thermal non-equilibrium denaturation experiments to study DNA point mutations. We show, that from the denaturation curves, we are able to detect the genotype of the target, in both homozygous and heterozygous cases with oligonucleotide targets (3). First results from PCR amplified DNA confirms the applicability of the setup for point mutation detection in biological samples. In this case, the temperature regulation is extremely useful, enabling hybridization under stringent conditions to overcome long ssDNA secondary structure.

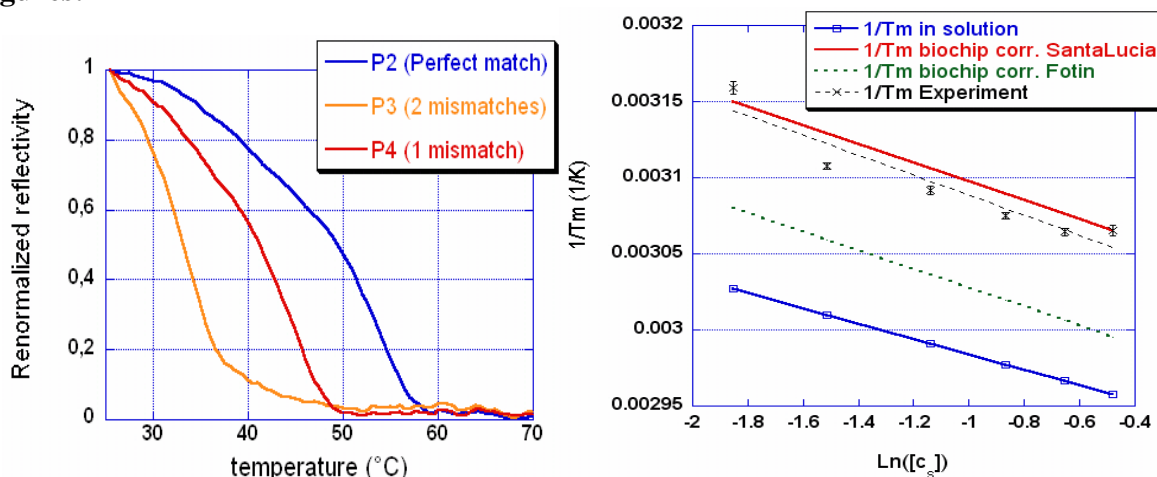
In a more fundamental approach, studies have been carried out to determine the influence of the salt concentration on DNA melting characteristics. While the melting temperature shows a dependency of the monovalent cation concentration as expected from DNA melting studies in solution, our experiments show, that no prediction is currently available that reliably forecasts the melting temperature for our 2D on-chip environment. To further analyze DNA denaturation in conditions readily applied in on-chip experiments, the influence of formamide on DNA thermal denaturation will be presented.

We further show the applicability of the system to the investigation of DNA lesion repair mechanisms. For this purpose, we address the DNA chip specifically with different lesions that can be studied in parallel using a sandwich system to guarantee full regeneration of the chip (4). Interactions of Fapy DNA *N*-glycosylase (FPG) and its human equivalent hOGG1 can then be followed by SPRi and characterized regarding kinetics and activity of the enzymes on all lesions present on the chip. In this way, for example, we are able to show, the covalent linking of FPG to 8-oxo-7,8-dihydro-2'-deoxyguanosine in the presence of NaBH₄, which is a direct proof of the enzyme activity. Our approach is a versatile layout that allows numerous applications for DNA repair studies.

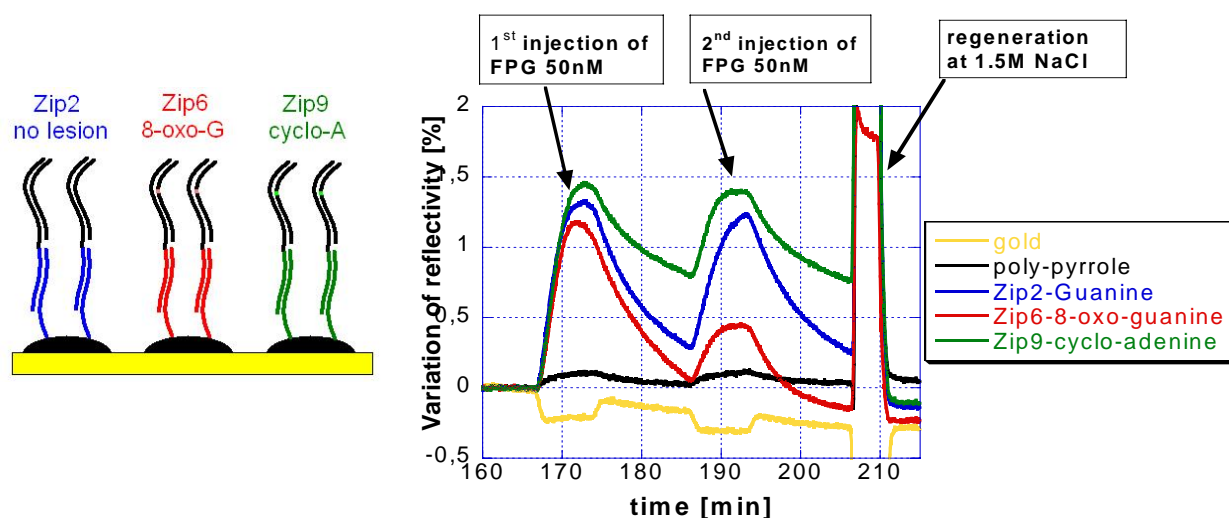
References:

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2. Fiche, J. B., A. Buhot, R. Calemczuk, and T. Livache. 2007. Temperature effects on DNA chip experiments from surface plasmon resonance imaging: Isotherms and melting curves. *Biophysical Journal* 92:935-946.
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Figures:



On the left: DNA thermal denaturation target T2 hybridizing perfectly to probe P2 and presenting one 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. **On the right:** 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.



Two consecutive injections of *N*-fapy-glycosylase (FPG) on DNA chip presenting different lesions, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-G) and (5'S)-5',8-cyclopurine-2'-deoxynucleoside (Cyclo-A), as indicated on the left. SPRI allows comparing affinities of FPG towards the lesions and reveals indirectly the cleaving action of the enzyme. The second injection shows a reduced binding specifically on 8-oxo-G spots, where the lesions is excised.