Kinetic discrimination of DNA single-base mutations with NPS

Insplorion's Localized Surface Plasmon Resonance (LSPR) technology empowers the discrimination of DNA strands differing by a single nucleotide through real-time kinetic measurement of hybridization to DNA probes on the sensor. The ratio between single-nucleotide polymorphism and perfectly complementary strands can also be inferred.

Introduction

A single-nucleotide polymorphism (SNP) is a variation of one nucleotide in a DNA sequence. SNPs represent the most abundant type of genetic variation found in nature and are one of the causes for differences in susceptibility to disease or response to treatments between two individuals. There are several SNPs with clinical prevalence and their detection can be critical for the selection of the correct treatment or in the research of disease mechanisms. Our NPS technology provides critical information on the kinetics of hybridization of DNA.

Experimental Procedure

Insplorion's bare gold nanodisc sensors were functionalized with thiolated oligonucleotides containing the probe sequence using a peristaltic pump at a constant flow rate. Different concentrations of samples containing a perfectly matching sequence (PM) and single nucleotide mismatched sequence(MM) were then injected into the system, either separately or in mixtures of different ratios. During each experiment, the samples were allowed to hybridize with the probe and the NPS signal was monitored until the reaction reached a steady-state.

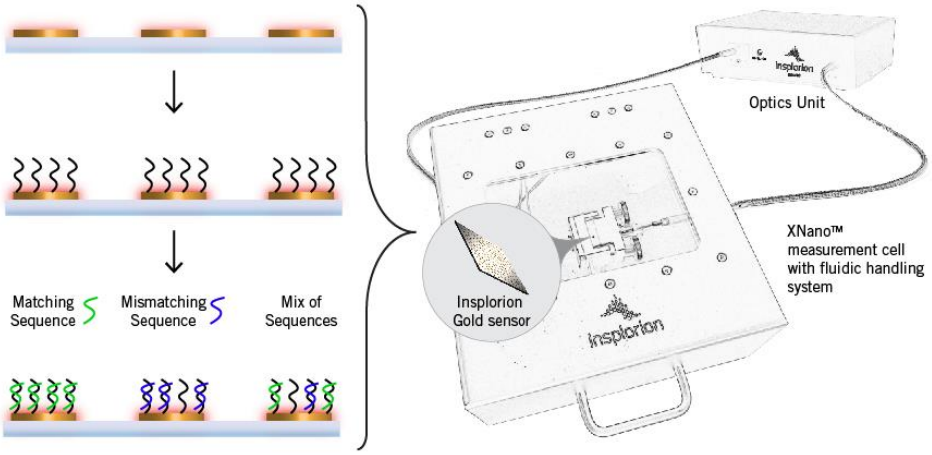

Figure 1: Insplorion system setup. The inset shows a schematic illustration of the sensors used in this application example (not to scale). The

Results

The kinetics of the surface functionalization was followed using Insplorion's XNano. The LSPR of Insplorion's sensors was measured during the functionalization process. The binding of thiol groups in the DNA probes changes the refractive index at the nanodiscs' surface, which is then translated into a shift in the plasmonic peak of the sensors. The position of the plasmonic peak gives information on the interactions between the surface and the samples. A plasmon peak shift of 1.1 ± 0.2 nm was detected and the surface density of probe DNA on the gold nanodiscs was estimated to be 2.8 ± 0.1 × 10^{12} molecules.cm^{-2} using a Langmuir kinetics equation with an association rate constant of 1.64 ± 0.02 × 10^{4} M^{-1}.s^{-1}.

The introduction of increasing concentrations of target DNA molecules resulted in a further shift of the NPS signal proportional to the concentration of target molecules at equilibrium. This allowed for the determination of the range of detection, and the subsequent calculation of a limit of detection of 10 nM and 12 nM for PM and MM, respectively. This sensitivity is in the same range obtained when using PCR and fluorescence methods.

The kinetics of the hybridization can also be investigated. The process can be treated as an association process in a pseudo-first

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order kinetic reaction, since the experiments were made under a large excess of target strands. The values of k_a were obtained from the pseudo-first order rate equation. Notably, the k_a obtained for the PM sequence ($1.46 \pm 0.06 \times 10^4$ $M^{-1}.s^{-1}$) was more than double that of the MM sequence ($4.5 \pm 0.2 \times 10^3$ $M^{-1}.s^{-1}$). The Insplorion XNano allowed for an easy discrimination of a single base in a 93-mer.

In clinical settings it is important to be able to detect point mutations in mixed samples. Thus, different ratios of PM and MM solution mixtures were tested on the same biosensors as the previous experiment and the LSPR peak was measured over time (Figure 2A). Since the sample was a mixture of targets, the kinetic parameter being measured is the apparent rate constant (k_{obs}) instead. k_{obs} presented a linear variation with the molar ratio of complementary and mismatching sequences (Figure 2B). This suggests that the presence of a mismatched sequence kinetically interferes with the complementary target's hybridization process, behaving as a competitor. On the other hand, a linear variation dependent on the ratio of the targets can potentially allow for estimating ratios in unknown samples.

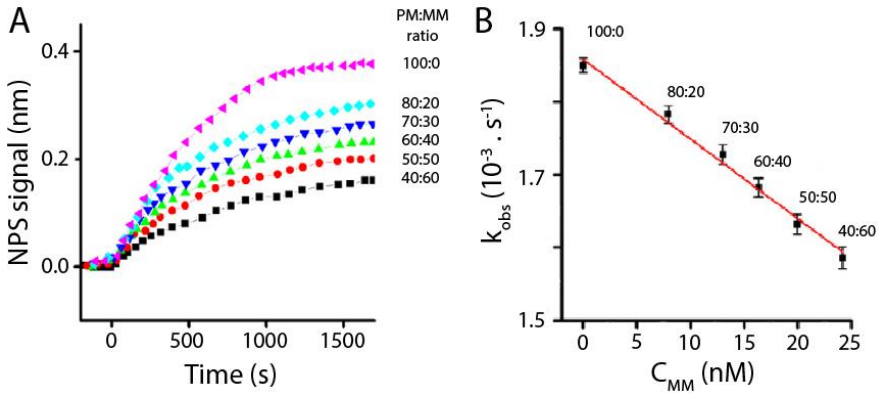

Figure 2: Kinetic analysis of two-component systems (PM and MM) interacting with surface bound probe molecules as a function of time for different PM:MM molar ratios. (A) The NPS signal as a function of time for the different molar ratios and PM at 40 nM as a reference. (B) Rate constants k_{obs} from the kinetic curves of Δλ (t) for PM pure solution and PM: MM binary solution versus MM target concentration (C_{MM}); the red solid line is the linear fit.

Conclusions

Insplorion's nanoplasmonic sensing technology is a unique method for studying the hybridization of DNA strands without labelling of any kind. The XNano is a valuable tool, not only for the detection of DNA, but especially for the understanding of the kinetics associated with DNA hybridization. The system proved to add unquestionable value in the determination of association constants, understanding the associated kinetics and even discerning ratios of complementary and mismatched sequences in the same solution. There is a clear potential to further develop the technique into a robust diagnostic system that is fast, label-free and presents similar sensitivity as traditional fluorescent and PCR-based techniques.

This study was originally performed by researchers at the Marletta group, Laboratory for Molecular Surfaces and Nanotechnology (LAMSUN), Department of Chemical Science, University of Catania and CSGI, Italy.

References

[1] *Kinetic discrimination of DNA single-base mutations by localized surface plasmon resonance, Antonino Rapisarda, Nicoletta Giamblanco and Giovanni Marletta, Journal of Colloid and Interface Science 487, 2017, 141–148*

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