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RAPID DISCRIMINATION OF SINGLE-NUCLEOTIDE DIFFERENCES THROUGH THE SPATIAL ANALYSIS OF A NANOFLUIDIC-EMBEDDED BIOSENSOR
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ABSTRACT
We demonstrate that the spatial analysis of nanofluidic-embedded biosensors permits the fast and direct discrimination of single-nucleotide difference (SND) within microRNA sequences in a single step interaction. We first show that the spatial hybridization profile depends on the duplex affinity, and we speculate that the affinity constant can be inferred during target injection, which is not possible in local analysis where the dissociation step through rinsing must be conducted. We finally manage to discriminate a GT mismatch on a microRNA sequence.

KEYWORDS: Single-Nucleotide Difference, MicroRNA, Nanofluidic, Biosensing, Fluorescence, Hybridization

INTRODUCTION
The gold standard for the detection of SND is the Next Generation Sequencing [1], a costly and time-consuming approach that enables the systematic discovery of new SND. Following identification, alternative target detection methods using an appropriate probe design can be relevant. Despite the fact that highly sophisticated detection methods have already been developed, none of them is compatible with direct detection and absolute quantification of SND since they often require a cumbersome, multistep protocol including target amplification [2-4]. Here, we present a novel nanofluidic-embedded biosensor, specific enough to detect SND via affinity discrimination in a single-step real-time detection protocol.

THEORY
Our nanofluidic platform is characterized by a short diffusion time through the channel (height ~500 nm) to the sensor surface and molecular interactions limited by the convection rate since the sensor length (~ 50 μm) is much larger than the channel height. As a result, unlike in the case of microfluidic devices, we observe the depletion of target molecules on the sensor surface along the fluid flow that leads to a non-uniform hybridization rate (figure 1a). The novelty of our approach is to study the hybridization along the sensor length (blue analysis in figure 1b), as opposed to the local hybridization which is solely limited by the reaction rate (classic kinetic analysis shown in red in figure 1b). We also exploit the other advantage associated to the nanoscale size of the channel: the low fluorescence background resulting from the reduced number of labeled targets in solution above the sensor permits the real-time measurement of hybridization rate by fluorescence microscopy without washing procedures.

Figure 1. a) Working principle of the nanofluidic biosensor: target molecules (green) injected in the nanochannel are trapped by the specific probes (blue) immobilized onto a gold patch. This results in a depleted zone and the concentration of target molecules decreases along the fluid flow. b) Time lap fluorescence images recorded onto the sensor (scale bar = 10 μm) and data analysis: classical kinetic study is obtained by plotting the fluorescence (one camera pixel) versus time (top, red), and profile analysis, obtained by plotting the fluorescence along a line of pixels on the sensor versus the distance (bottom, blue).
RESULTS AND DISCUSSION

We report that the hybridization profile along the sensor length as a function of time is highly dependent on the affinity of the duplex. We have performed hybridization experiments at different temperatures to fine tune the affinity of two different microRNA targets to the same probe sequence. The resulting plots using the local and global analysis show that discrimination can be achieved using both approaches (figure 2a-b). However, while the sensor wash is absolutely necessary to enable target discrimination during dissociation with the classical local analysis, a difference in hybridization profiles is clearly observed after a given time of reaction without requiring any dissociation phase, thus saving assay time and gaining in simplicity.

By optimizing the interaction temperature and the probe design, we finally demonstrate that our platform can identify one of the most stable mismatch (GT) on a 22 bases single stranded RNA sequence after a few minutes of interaction in a single step protocol (figure 3).

CONCLUSION

Taken together, we devised during this study a highly specific, real time identification method of single base nucleotide difference that holds great promise for the study of microRNA role and function in e.g. cancer research. Furthermore, this work opens doors to any transduction scheme for spatially resolved biosensors integrated into nanofluidic channels for applications where high-selectivity and short analysis times are required.

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