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Grant Agreement no. 829010

PRIME

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DELIVERABLE 2.4:
First printed sensing functions (public version)

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Responsible partner: CSIC
Report Author(s): Gabriel Alfranca, Jesús Martínez de la Fuente, Carlos Sánchez Somolinos
Type1[1]: R
Dissemination Level[2]: PU

[1] **Type**: Use one of the following codes (in consistence with the Description of the Action):
R: Document, report (excluding the periodic and final reports)
DEM: Demonstrator, pilot, prototype, plan designs
DEC: Websites, patents filing, press & media actions, videos, etc.
OTHER: Software, technical diagram, etc.

[2] **Dissemination level**: Use one of the following codes (in consistence with the Description of the Action)
PU: Public, fully open, e.g. web
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CI: Classified, information as referred to in Commission Decision 2001/844/EC

CSIC Consejo Superior de Investigaciones Científicas · TU/e · Max-Planck-Gesellschaft · Universidad Zaragoza 1542 · BNN · BEOnChip Biomimetic Environment On Chip

DELIVERABLE D2.4: First printed sensing functions

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1. DOCUMENT HISTORY

Version	Date	Authors/ who took action	Comment	Modifications made by
0.1	23.01.2021	Gabriel Alfranca, Jesús Martínez de la Fuente, Carlos Sánchez Somolinos (CSIC)	First draft sent to PIs	
1.0	28.01.2021	Carlos Sánchez Somolinos (CSIC)	Submitted to Commission	

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2. RESULTS AND OUTLOOK

PRIME targets to integrate new ultra-sensitive and selective sensors in the chip and readable with light. The final device will be remotely addressed and read using simple photonic elements that can be integrated in compact, portable, and low-cost operation-and-read devices.

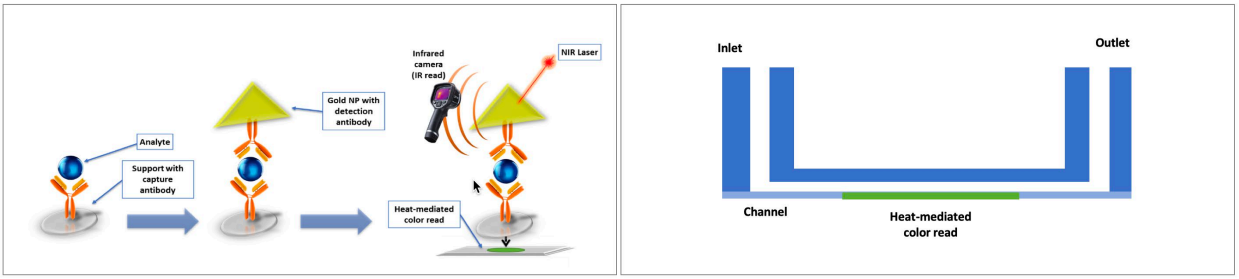

Figure 1, (left) PRIME nanoparticle-based sensor with a colorimetric thermal transducer and (right) channel-like sensor configuration.

In the course of this task we have been progressing on the design and preparation of the PRIME sensing function. The preparation of the channel structures within a microfluidic chip has been undertaken and functionalization protocols developed in WP1 have been adapted to provide the channel with analyte-capturing antibodies (Figure 1). This channel in combination with the plasmonic nanoparticles provided with detection antibodies can lead, in the presence of an analyte, to heat that can change the colorimetric thermal transducer (CTT), which is the optical detection element.

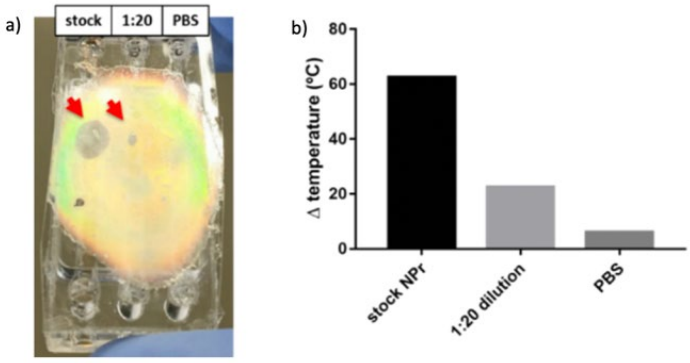

Figure 2, Irradiation of the channels: a) result of the irradiation of the channel prototypes with two dilutions of nanoparticles and a nanoparticle-free control (PBS); b) graphical representation of the temperature registered in each channel after 2 min irradiation (n = 1).

After testing several prototypes of channels and discerning the most optimum design, we integrated the CTTs in the microfluidic chip (Figure 2). These prototypes, when loaded with a dilution of nanoparticles and exposed to the NIR laser, are able to heat enough to produce a visual change in the CTT. The reflection optical appearance of the film changes in the proximity of the

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irradiation area, revealing a correlation between the concentration of nanoparticles and the area in which the color disappears, which in turn corresponds to a higher increase in the local temperature.

In addition, the results have demonstrated our capability to produce biofunctional channel prototypes that are able to bind antibodies for the generation of a significant heat signal detected with an IR camera. The nanoparticles conjugated with antibodies specifically bind to the analyte to form a sandwich with said capture antibody, and upon irradiation they are capable to produce a detectable signal. Three methods of biofunctionalization were selected (Figure 3) and tested in 96 well plates as well as in the channel prototypes. From these, one method produced a significant heating signal in the prototypes and thus was selected for future experiments and optimization.

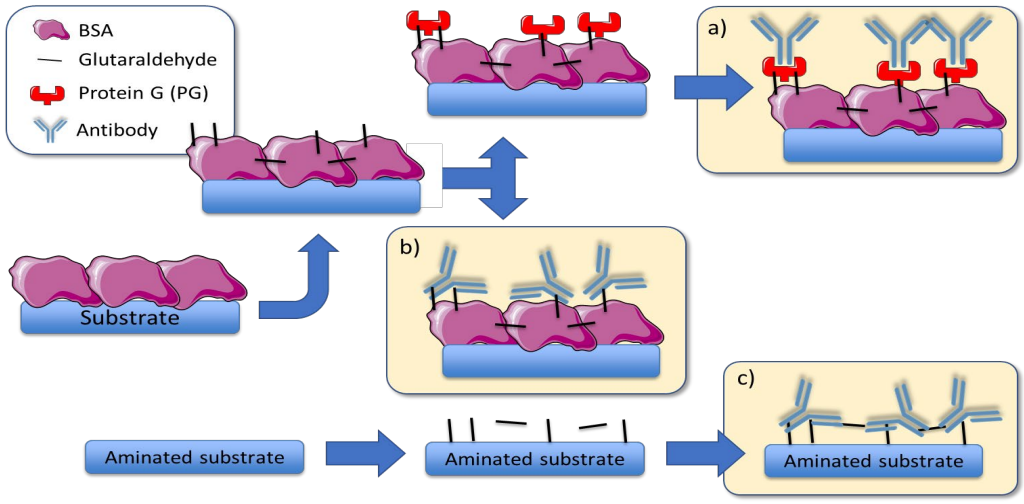

Figure 3, Schematic representation of the different approaches of the conjugation of the capture antibody on the substrate: a) corresponds to the oriented specific interaction of the antibody using Protein G crosslinked on a BSA layer; b) consists in the crosslinking of the antibody directly on the BSA layer, without using Protein G; and c) resorts to a substrate containing amino groups which are then used to crosslink the antibody directly on it, without using a BSA layer.

The main remaining challenge at this stage would be to produce enough heat on the channels to be able to induce a change in the CTT. Currently, the heat generated by the immunoassay is much lower in the chips than the one observed in the 96 well plates. The temperature change observed in the channels is significantly higher than the controls when measured with the IR camera, but not enough to induce a change in the optical appearance of the CTTs.

There are several approaches that could be applied to overcome this challenge. By changing the type of CTT that could induce a visible change in their optical appearance at lower temperatures. In addition, the interaction of the capture antibody with the substrate of the channel could be improved by optimizing the method of crosslinking said antibody on the aminated substrate. Furthermore, the optimization of the conditions of the immunoassay are necessary, which could allow for a higher concentration of nanoparticles retained in the channel and the subsequent increase in the heat signal produced. Finally, the signal generated could also be improved by optimizing the design of the chip.

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In the next months we will select the most ideal strategy among these and start an iterative process of optimization of the assay with the main aim to improve the heat signal and/or to generate more sensitive colorimetric thermal transducers.

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