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PRIME

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DELIVERABLE 1.5:
Sensing printable materials

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Responsible partner:	TU/e
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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
- CO: Confidential, restricted under conditions set out in the Model Grant Agreement
- 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 D1.5: Sensing printable materials

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1. DOCUMENT HISTORY

Version	Date	Authors/ who took action	Comment	Modifications made by
0.1	20/07/2021	DJM	First draft sent to WP1 PIs	DJM / GAR/ JMdlF/ CSS
0.2	29/07/2021	DJM	Second draft sent to PIs	DJM / JMdlF / CSS
0.3	30/07/2021	DJM	Third draft sent to CSS	DJM / CSS
1.0	30/07/2021	CSS	Submitted to Commission	

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2. RESULTS AN OUTLOOK

Microfluidic devices manipulate tiny amounts of fluid enabling cost-effective, fast, accurate and high throughput analytical assays. Progress in Microfluidics has huge impact in environmental pollution monitoring, biohazard detection and biomedicine, contributing to the development of new tools for drug screening, biological studies, point-of-care diagnostics and personalized medicine. Despite this huge potential, Microfluidics market growth is heavily constrained by the complexity and high prices of the required large-scale off-chip equipment and its operational cost.

Sensor technologies have attempted the use of nanoparticles (NPs) containing fluorescent or colorimetric markers. However, the major limitation of nano biosensors based on color/fluorometric transducers are their low sensitivity, and the presence of false positives induced by another colorant presented in the sample matrix. Usually, complex sample treatments are required to eliminate interferences. This increases the complexity and duration of the assay. These limitations are not present in thermal transducer biosensors, simplifying (or even eliminating) any sample treatment.

PRIME targets to implement and integrate through printing of new ultra-sensitive and selective sensors embedded 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.

The sensors developed within PRIME is intended to be a bioassay built-up over a colorimetric thermal transducer (CTT). This sandwich assay comprises the immobilization of the antibody on a CTT, then the addition of the sample containing the antigen, and finally, the addition of the bioconjugate, which is the resulting molecule after functionalizing the gold nanoprisms (AuNPs) with the antibody pair. The detection step comprised the irradiation of the biosensor with a laser and the visual detection of the temperature increase using the CTT (Figure 1).

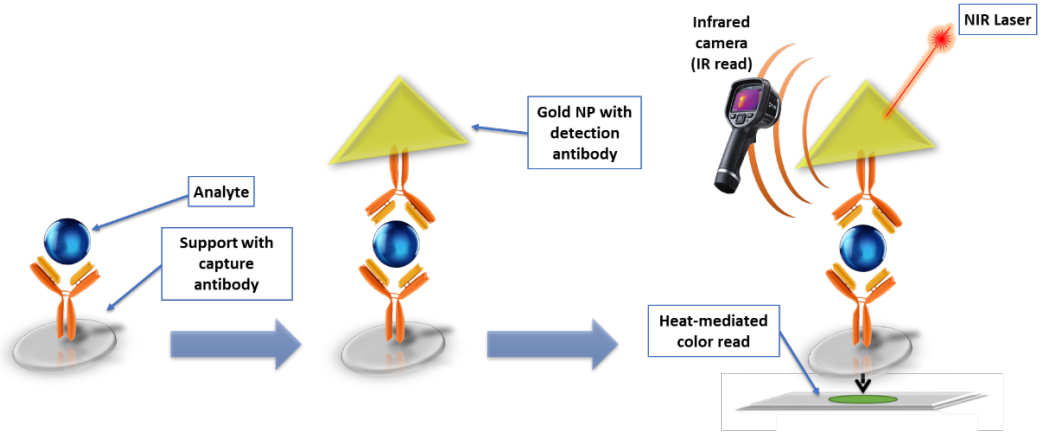

Figure 1. Scheme of the design of the assay in its current form after its optimization.

In the course of this task sensing materials and their processing have been investigated, seeking for a new generation of selective and ultrasensitive nanoparticle-based sensors. First sensing materials have been developed together with protocols for their implementation in the chip.

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Jettable CTT inks have been formulated, various organic-based compositions studied, and the sensitivity optimized. Furthermore, we have been looking into alternative materials that can be printed to produce robust sensing layers with a more accurate read-out.

In addition, we synthesized gold nanorods (AuNR) and compared them with nanoprisms (AuNPr). The synthesis of AuNPr consists of a one-pot method,[1] which is based on the reduction of the gold salt using sodium thiosulfate, which also acts as a surfactant, in presence of iodide ions, both of which are added into sequential additions to the dilution containing the gold salt. In the case of gold nanorods (AuNR), the synthesis is based on a seeded-growth method[2] which resorts to the use of Hexadecyltrimethylammonium bromide (CTAB) as surfactant and a two-pot process consisting of the generation of small gold seeds (~3 nm). These seeds are then added into a reduced gold solution ("growth solution"), which kicks off the growth of the seeds into cylinder-shaped nanoparticles.

Both types of nanoparticles showed high absorptivity in the NIR range (Figure 2). These optical properties allow them to convert light energy into heat by the photothermal effect when irradiated with a laser-centered around their localized surface plasmon resonance (LSPR).

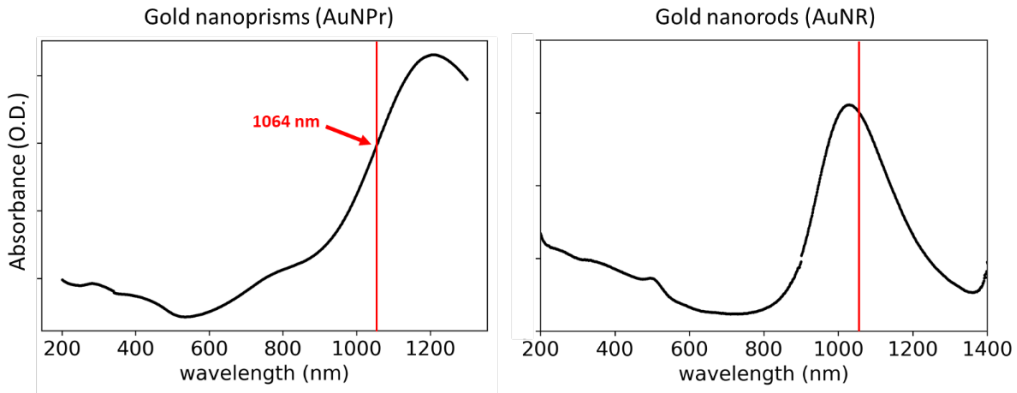

Figure 2, UV-Vis-NIR spectra of AuNPr and AuNR. The red line represents the location where the NIR laser is centered.

The heat characterization revealed that the AuNR are slightly better heat transducers than the AuNPr when measured at the same optical density. In fact, after the entire characterization and comparison of their properties, both types of nanoparticles were determined to be suitable for the tasks ahead. However, gold nanoprisms show better potential in terms of scalability, ease of synthesis, and biocompatibility (no need for toxic surfactants).

The optimization of several steps of the bioconjugation protocol was carried out. It is important to block properly the nanoparticle after adding the detection antibody to avoid unspecific interactions with the real sample. Two blocking agents were chosen, namely bovine serum albumin (BSA) and polyethylene glycol (PEG). The results indicated that PEG acts better as a blocking agent than BSA.

The nanoparticles were successfully bioconjugated with an antibody anti-CEA (carcinoembryonic antigen) to serve as detection analyte, which was demonstrated in both direct and sandwich thermoLISA experiments, validating the assay.

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Future experiments will move towards the development of a chip prototype optimized for its bioconjugation with a model antibody anti-CEA, forming the sandwich in the chip, and using the CTT to detect the heat generated by a visual change in color.

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3. REFERENCES

1. Alfranca, G. *et al.* Gold nanoprism-nanorod face off: Comparing the heating efficiency, cellular internalization and thermoablation capacity. *Nanomedicine* **11**, 2903–2916 (2016).
2. Vigderman, L. & Zubarev, E. R. High-yield synthesis of gold nanorods with longitudinal SPR peak greater than 1200 nm using hydroquinone as a reducing agent. *Chem. Mater.* **25**, 1450–1457 (2013).

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