# Advancements of the biosensing technologies for the preliminary biomarkers

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## **STRATEGY OF THE PROJECT**

**1**a

New biomarkers

discovery and

validation





technological platform for the detection of the detection of "known" "known" biomarkers

selected biomarkers and selection of the "easiest" to detect

technological platforms to the final biomarkers and validation on art. samples/

samples and validation in the clinic

Phenylalanine

m.w. 165.19 g/mol

Phe+

**Gold nanoparticle** 

(AuNP)

ΌΗ

Quinolinic and Kynurenic acids

m.w. 189.17 g/mol



Still running

#### m.w. 167.12 g/mol



• The molecules (template) are used to synthetize a MIP on an electrode.

mechanisms of

biomarkers

- The cavities obtained after the removal of the template from the polymer are "specific" to the template.
- The current recorded with electrochemical measurements in a redox solution is hindered in presence of the target.
- The current reduction is proportional to the target molecule concentration in the sample.

- Lateral Flow Assays (LFAs) typically use antibodies as bioreceptors, but this is an issue when the target is a small molecule.
- We designed a LFA based on an aptamer i.e. a short DNA sequence with a conformation allowing the  $\bigcirc$ binding of Phenylalanine (Phe).
- The binding of Phe releases an AuNP labelled sequence Sample of DNA. ОН ИН
- The assay can be performed as a displacement or a competitive 🖓 👫 🕂 assay.
- **Displacement assay**

**Competitive assay** 

Sample

Phe+ Phe-



ET << ET'

Current

E/V



+ Target Signal-ON

Potential (V vs Ag/AgCI)

ignal-OFF

Template removal

- Inkjet printed electrodes are functionalized with aptamers labelled with a methylene blue (MB) redox probe.
- The aptamers conformation changes upon NGAL binding and the distance between MB and the surface change as well.
- The different distance causes a variation of the electron transfer between the probe and the electrode surface, thus of the recorded signal, proportionally with the NGAL concentration.

#### • We developed Near Field Communication (NFC) inkjet printed antennas for the wireless readout of our biosensors.

- The fabrication is easy and straightforward and the biosensors can be printed together with the antenna.
- The smartphone app allows both impedimetric and amperometric measurements.

### Wireless smartphone readout



**CONCLUSIONS** 

Time

Step size

Amplitude

- The technological platforms for the "known" biomarkers, i.e. Kynurenic acid, Quinolinic acid, Phenylalanine, and NGAL are at an advanced level and will be ready in few months.
- Innovative methods of biosensors fabrication, wireless readout at the point of care, and smartphone based data acquisition and analysis have been introduced and are ready to use. A new app will be soon developed thanks to a company and will be designed to be easy to use and effective.
- The list of the new validated biomarkers is urgently needed to define the final ones to adapt/redesign the developed detection platforms.

## ACKNOWLEDGMENTS

We acknowledge the great work and support received by the group of Prof. Ozcan for the MIP system development, the support of our former group member Claudio Parolo for the development of the Phenylalanine biosensor.



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## **STRATEGY OF THE PROJECT**

**1**a



**CROB-PREDICT** 



### Neutrophil Gelatinase-ass. Lipocalin (NGAL)

- SWV needs optimization of the parameters (amplitude and frequency) to obtain significant variations related to the aptamers conformations.
- The optimization consists in performing tests with and without a fixed concentration of NGAL in the chosen buffer/matrix setting a series of parameters' values on at least 4 functionalized electrodes.
- The measurement's order partially influence the result: many tests!





## CONCLUSIONS

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Inkjet-printed gold

electrodes (produced

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